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EDITORS :—

Dr C. D. DARLINGTON
John Innes Institution
Bayfordbury, Hertford, Herts.

Prof. R. A. FISHER
Whittingehame Lodge
Cambridge

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HEREDITY VOLUME 3 PART 3 DECEMBER 1949

# NUCLEAR AND CYTOPLASMIC INHERITANCE OF RESISTANCE TO INFECTION BY NODULE BACTERIA IN RED CLOVER

By P. S. NUTMAN

*Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, Herts.*

Received 19.xi.48

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## 1. INTRODUCTION

Nodule formation in the Leguminosæ is of practically universal occurrence. Of some 12,000 known species of legume only a few species in the Cæsalpinioideæ and Mimosoideæ groups appear to form no nodules, and palæntological evidence (McMillan, 1892, and Sargent, 1892, quoted by Fred, Baldwin and McCoy, 1932) suggests that the absence of nodules in these few exceptional species is the survival of a primitive condition. Outside the Leguminosæ nodules which contain actinomycetes occur on *Alnus*, and bacterial root nodules on certain Zygophyllaceæ (Sabet, 1946). Otherwise with a few doubtful exceptions the whole of the plant kingdom is devoid of root nodules. Mycorrhizal associations on the other hand are everywhere common—as they also are among legumes.

Within the Leguminosæ conditions of partial resistance occur: all strains of nodule bacteria have become specialised to form nodules on a limited number of species of host plant. So far about 20 groups of strains of *Rhizobium*, some recognised as species by some authors (Fred, Baldwin and McCoy, 1932), have been distinguished depending upon the range of host plant species each is able to infect. This cross-inoculation group specificity is not rigidly defined (Wilson, 1939; Kleczkowska, Nutman and Bond, 1944), and the groups are principally a convenient way of classifying strains. The exceptions to this classification most frequently occur, however, among groups like the pea and clover groups of bacteria which are antigenically related (Kleczkowski and Thornton, 1944).

There are thus two forms of resistance already known in nature; *viz.* (i) the total resistance of *Cassia spp.*, etc., to infection by all nodule

bacteria, and (ii) the resistance shown by plants of one cross-inoculation group to infection by bacteria isolated from other such groups. Further, failure in nodule formation can also arise by mutation of the bacteria to an avirulent form ; clearly, however, this does not involve the plant response and is not a resistance phenomenon.

A preliminary note on the discovery in red clover of an hereditary resistance to infection by its own strain of nodule bacteria was published in 1946 and in the present paper the complete results are presented.

## 2. MATERIALS AND METHODS

The original material was obtained from a commercial sample of Montgomeryshire late-flowering red clover (a diploid species) and all subsequent work was done within this variety with the exception of the original open pollination which was to material of unknown origin.

The plants were grown under standardised conditions which enabled bacteriological control to be maintained. The details have been described elsewhere (Thornton, 1930), the method consisting essentially of the culture of individual plants in test tubes ($6'' \times \frac{3}{4}''$) on slopes of previously sterilised agar medium which contains mineral salts but lacks combined nitrogen.

This method although unnecessarily complicated for the study of resistance *per se*, was used in order to determine the response of nodulating plants in segregating families. After a period of 3-4 months growth selected plants were potted into soil and were hand pollinated using Williams' method (Williams, 1925).

Hand harvesting resulted in a high proportion of " hard " seed. Softening of the seed coat and surface sterilisation was done in one operation. This consisted of submersion in concentrated sulphuric acid for 30 minutes followed by from six to ten washes in sterile water. This method for seed sterilisation has proved to be more satisfactory than any of the others more usually employed. No injury such as frequently happens with mercuric chloride, even when used without pretreatment, follows this method. Imbibition is uniform and immediate, and germination rapid ; the radicle emerging within about 6 hours of treatment.

Degrees of hardness varies individually ; half an hour's treatment completely eliminates hardness in all but a few families and is not long enough to damage the material ; damage follows from 1-3 hours' treatment. (This method has been found to be applicable to lucerne, vetch, lupin and radish but not to lettuce, barley or spruce.)

## 3. INHERITANCE OF RESISTANCE

### (*a*) *Source of material and original crosses*

The original resistant plant proved to be wholly resistant to infection by the infective strain A, and also to a collection of other

strains inoculated at a later stage, and to casual contamination occurring after the plant had been transferred to fresh soil. The phenotypic character was equally definite in derived resistant plants.

The original resistant plant after open-pollination gave resistant and susceptible progeny (abbreviated in tables and figures as *res* and *sus*). The resistant plants were chlorotic and capable of only feeble growth, few surviving to maturity.

These resistant plants (indicated in tables 1 and 2 as $F_1$ *res*) were crossed with susceptible sisters and half-sisters and also with unrelated susceptible plants, with the results shown in table 1.

TABLE 1

*Proportion of resistant progeny in reciprocal crosses of resistant $F_1$ plants with related and unrelated susceptible plants*

(*sus*, susceptible plant ; *res*, resistant plant)

| Related | | |
|---|---|---|
| *res* ♂ | *res* ♀ | |
| *sus* ♀ | *sus* ♂ | |
| 54·9 per cent.<br>4 families (82 plants) | 45·4 per cent.<br>4 families (66 plants) | |
| **Unrelated** | | |
| *sus* A or B ♀ | *sus* A ♂ | *sus* B ♂ |
| 0 per cent.<br>27 families (550 plants) | 0 per cent.<br>5 families<br>(138 plants) | 13·4 per cent.<br>heterogeneous<br>22 families<br>(164 plants) |

The crosses of *related* susceptibles with resistants gave equal numbers of resistant and susceptible progeny in families which were homogeneous and showed no reciprocal differences. The *unrelated* susceptibles were of two kinds *A* and *B*, *sus A* giving no resistant progeny and *sus B* giving a proportion of resistant progeny but only in the cross in which the resistant plant functioned as the ♀ parent. There is thus, it seems, a total elimination of resistants in the crosses of unrelated *sus B* ♀×*res* ♂ and a partial elimination in the reciprocal cross.

These results suggest that resistance depends on a Mendelian recessive and also on an agent transmitted more effectively through the ♀ parent ; the effect of this agent giving heterogeneous results in the mixed class.

This interpretation was confirmed by the results shown in table 2. Susceptible progeny of any cross listed in table 1 when intercrossed

TABLE 2

*Proportion of resistant plants in crosses involving the parents and progeny of families listed in table 1*

| Family no. | Cross | *sus* | *res* | $\chi^2$ |
|---|---|---|---|---|
| ... (>100) | UNRELATED *sus* parent (A or B) *inter se* | >1000 | 0 | ... |
| 9-15 (7) | RELATED *sus* of any origin *inter se* . | 325 | 111 | 0·049 (3 : 1) |
| 1- 4 (4) | RELATED *sus* of any origin × *res* parent | 73 | 75 | 0·027 (1 : 1) |
| 86 (1) | *res* parent *inter se* . . . . | 0 | 19 | ... |
| ... | *res* progeny of *sus* B × *res* $F_1$ ♀ . . | no material | | |

gave 25 per cent. resistants and when backcrossed to resistant parents gave 50 per cent. resistants. The single cross between the two resistant parent plants bred true for resistance and the unrelated susceptible parents bred true for susceptibility ; see also text-fig. 1, page 283.

The resistant progeny of the *sus* $B \times res$ $F_1$ ♀ (table 1) were very feeble and could not be bred from, so that the resistant line had to be revived by again crossing to unrelated susceptibles : this will be distinguished as " outcrossing " in the following discussion. Outcrossing is also required by the incompatability relationships in red clover. The recessive resistance factor revealed in these preliminary crosses will be designated *r*.

The interaction between the gene and the maternally inherited component of resistance will be discussed after further evidence for the existence of this gene, and for the maternal inheritance have been separately considered.

### (*b*) *The gene r*

The preliminary crosses showed that the original outcrosses were of two kinds ; between the original resistant plant *rr* and homozygous *RR* plants, and between the original resistant plant and heterozygous *Rr* plants.

It is not legitimate, therefore, to refer to the descendant families of the original resistant plant by filial generation number. Instead, in table 3 which shows the segregation of the gene *r* in a large number of families of very diverse origin, the crosses are simply classified by parental and grandparental phenotypes. Thus families numbered 1 to 8, and 41 and 42 consist of crosses between susceptible and resistant plants and the remainder of crosses among susceptible types ; and in families 1 to 8 and 9 to 40 resistant plants occur as immediate ancestors of the susceptible parents of the crosses listed, and in the remaining crosses both grandparents, at least on one side, were themselves also susceptible. No differences between reciprocal crosses were observed.

In the families (1 to 40) of varied origin falling outside the secondary division in the table; *i.e.* with at least one resistant grandparent, very good agreement exists between observed and

TABLE 3

*The segregation of susceptible and resistant plants among the descendants of the original outcrossed resistant plant*

| Family nos. | Type of cross | Phenotypes of grandparents | Segregation | | | | $\chi^2$ | P< |
|---|---|---|---|---|---|---|---|---|
| | | | Observed | | Expected | | | |
| | | | *sus* | *res* | *sus* | *res* | | |
| 1- 8 (8) | *res*×*sus* | *res*; *res* and *sus* | 103 | 105 | *104* | *104* | 0·019 | ... |
| 41 (1) | ,, | *res*; *sus* and *sus* | 50 | 2 | *26* | *26* | 44·31 | 0·01 |
| 42 (1) | ,, | ,, | 59 | 0 | ... | ... | ... | ... |
| 9-37 (29) | *sus*×*sus* | both *res* and *sus* | 1075 | 352 | *1070·2* | *356·8* | 0·08 | ... |
| 38 | ,, | *res*; *res* and *sus* | 40 | 5 | *33·8* | *11·2* | 4·63 | 0·05 |
| 39, 40 (2) | ,, | (selfs) *res* and *sus* | 1 | 1 | *1* | *1* | ... | ... |
| 43-47 (5) | ,, | *res* and *sus*; *sus* and *sus* | 164 | 12 | *132* | *44* | 31·03 | 0·01 |
| 48-54 (7) | ,, | ,, | 107 | 0 | ... | ... | ... | ... |
| 55-63 (9) | ,, | both *sus* and *sus* | 173 | 24 | *147·8* | *49·2* | 17·26 | 0·01 |
| 64-85 (22) | ,, | ,, | 467 | 0 | ... | ... | ... | ... |

expected values; in one family only (family 38) is there an excess of susceptible plants. The families within each type of cross are, as we shall see, generally homogeneous.

In the remaining families, in which the susceptible parent plants were themselves derived from susceptible plants the families are heterogeneous. The proportion of mixed families (15 families in 43) is in accordance with expectation; a closer study of the lineages showed that 14 families should have been mixed. As well as being heterogeneous the proportion of resistant plants in these families departs significantly from the expected values. This discrepancy may be due to (1) linkage with lethal genes, (2) depression of the low vitality of the resistant plant on inbreeding, or (3) the dilution of the cytoplasmic factor of the inheritance.

The last possibility (3) affords a possible explanation if the production of the cytoplasmic material is under the control of the gene *r*. As noted above, one or both grandparents of the susceptible parents of the abnormally segregating families were susceptible so that the line of descent was wholly susceptible for two generations. Under these conditions dilution of the cytoplasmic factor could occur, and with insufficient of this material the homozygous recessives would be eliminated (see table 1). The breeding system of red clover makes

the detection of zygotic lethals uncertain and little difference was noted in the seed set in normally segregating families (46·0 per cent.) and the abnormally segregating families (43·5 per cent.). The figures for germination, however (85·2 and 73·3 per cent. respectively), do suggest an elimination of about the proportion required. The discrepancy in the segregating families and the zygotic and pre-zygotic elimination both amount to about 15 per cent.

### (c) *Segregation within the resistant lines*

The breeding behaviour of resistant plants is shown in table 4. All progeny of the single successful cross between resistant plants

TABLE 4

*The aberrant segregation of susceptible plants within the resistant lines*

*Cross type* res × res

| Family no. | Number of *sus* and *res* ancestors | | Number of different outcrosses in ancestry | Segregation | |
|---|---|---|---|---|---|
| | *sus* | *res* | | *sus* | *res* |
| 86- 89 (4) | 0- 3 | 4- 6 | *2* | 0 | 68 |
| 90- 99 (10) | 5-20 | 7-20 | *3-8* | 0 | 207 |
| 100-104 (5) | 5-10 | 5-10 | *3-5* | 10 | 201 |
| 105-108 (4) | 8- 9 | 7-10 | *3-5* | 40 | 46 |

derived directly from the original resistant plant were resistant (family 86). This cross was highly infertile and the seed poorly viable and the plants dwarf and abnormal. From approximately 400 florets pollinated, only 19 seedlings were obtained ; other crosses of this type were completely infertile.

Other crosses between resistant plants yielded larger families ; most of these bred true for resistance but some gave rise to susceptible plants. These aberrant susceptibles on which the first nodules developed normally at the 2-3 leaf stage, were not due to pollen contamination since control selfings remained normally sterile.

The unexpected occurrence of these susceptible plants in what should be pure breeding *rr* lines may have been due, failing back mutation, to the dilution of the cytoplasmic factor, or to modifying factors or to both. The dilution of a cytoplasmic factor is difficult to envisage in these families since it is presumably under genic control and would be continually produced in the resistant parent plants. Further, the occurrence of susceptible plants bears no relation to the proportion of susceptibles in their recent ancestry, and it does not increase regularly on inbreeding as would be expected on a dilution hypothesis or if due solely to modifying factors. An alternative explanation is, however, suggested by the distribution of these susceptible plants within the resistant lines. Table 4 shows that anomalous

susceptible plants occur only when the original susceptible parents were likely to be different plants. It has been assumed that the original casual outcross was to a number of plants, as was most probably the case, so that the minimum number of outcrosses is two. In the pedigrees of the anomalously segregating families the number of outcrosses always exceeds two. This relationship obtains whether or not resistant plants are found in the progeny of the outcrosses themselves.

This suggests that some of the outcross material is heterozygous for different resistance factors; the varying ratios indicating that some of these are simple and others more complex. In the families 99, 100 and 105 one parent was itself a resistant daughter plant of an outcrossed resistant plant (family 138, table 6), and may therefore be expected to have been heterozygous for one complementary factor only; an approximately equal number of resistant and susceptible plants occur. The parents of family 94 may also have been heterozygous for one additional factor only, and are in fact also related to family 138.

On this view "aberrant" susceptible plants would therefore be double heterozygotes and on crossing with normal $r_1r_1$ $R_2R_2$ plants will give a 1 : 1 ratio and when crossed amongst themselves a 9 : 7 ratio. In table 5 data from crosses in which these aberrant susceptible

TABLE 5

*The breeding behaviour of aberrant susceptible plants* (ab. sus) *segregating from pure* res *parents*

| Family no. | Type of cross | Observed segregation | | Expected segregation | | $\chi^2$ |
|---|---|---|---|---|---|---|
| | | *sus* | *res* | *sus* | *res* | |
| 001-004 | *Ab. sus* × *res* | 74 | 63 | *68·5* | *68·5* | 0·883 |
| 005-008 | *Ab. sus* × *sus* | 18 | 14 | *16·0* | *16·0* | 0·500 |
| 009-012 | *Ab. sus* × *ab. sus* | 15 | 7 | *12·4* | *9·6* | 1·249 |

plants are involved support this interpretation. The data in table 5 also agree fairly well with the assumption that the aberrant susceptible plants are heterozygotes produced by back mutation; $\chi^2$ for the last two group of families being 6·00 and 0·54. This explanation fails, however, to relate the occurrence of these 4 families to the outcross family 138.

With regard to the remaining families in table 4 in which only a few aberrant susceptible plants appear, the proportions of susceptibles to resistants (10 : 201) corresponds to a 1 : 16 ratio ($\chi^2$, 2·016). This suggests that in these families the effect of the resistance factor may be overcome by a pair of modifying recessive factors. Additional data is clearly needed to substantiate these hypotheses.

### (*d*) *Investigation of maternal inheritance*

The recessive gene for resistance is fairly common in the heterozygous condition in the wild population but its expression in the homozygous form is rare on account of its dependence on the maternally transmitted component which is also rare. The maternal effect will be denoted in the following discussion as being due to a specific substance probably of cytoplasmic origin which will be designated $\rho$.

In table 6 the regular manifestation of maternal inheritance in the outcrosses of resistant plants is demonstrated. In each generation

TABLE 6

*The maternal component in the inheritance of resistance*

The segregation of resistant plants in outcrosses of resistant plants to unrelated susceptible plants.

| Family no. | Cross ♀ ♂ | Number of generations separating resistant parent from original resistant plant | Segregation | |
|---|---|---|---|---|
| | | | *sus* | *res* |
| Original outcross | *res* × *sus* | ... | *132* | *17* |
| 108-112 | *res* × *sus* | 1 | *120* | *18* |
| | recip. | 1 | 164 | 0 |
| 113-135 | *res* × *sus* | 1 | *325* | *0* |
| | recip. | 1 | 386 | 0 |
| 136-138 | *res* × *sus* | 2 | *92* | *4* |
| | recip. | 2 | 65 | 0 |
| 139-144 | *res* × *sus* | 2 | *151* | *0* |
| | recip. | 2 | 133 | 0 |
| 145-148 | *res* × *sus* | 3 | *204* | *4* |
| | recip. | 3 | 77 | 0 |
| 149-153 | *res* × *sus* | 3 | *92* | *0* |
| | recip. | 3 | 101 | 0 |

the outcrosses were to susceptibles of two kinds (*a*) those which yield no resistant plants in their progeny irrespective of the direction of the cross, and (*b*) those which give segregation of resistant plants (the susceptible plant acting as paternal parent only).

This division may not always reflect differences in genotype since the ratio of susceptible to resistant plants in the segregating families does not conform to the expected value of 1 : 1. This ratio increases from 132 : 17 in the original outcrosses (including families which may or may not have given a segregation) to a maximum in the last set of outcrosses of 204 : 4. Within each group of outcrosses to known susceptibles the proportion of the two types are respectively 16 : 11, 12 : 1, 47 : 3, 30 : 1 ; and 19 : 2, 31 : 1, 42 : 1 ; and 33 : 1, 51 : 1, 58 : 1, 62 : 1, and thus form series asymptotic to the expected 1 : 1 ratio at the original outcross. This result suggests that in the original outcross generation a ratio of 1 : 1 may have obtained. The falling off of the ratio may be due to an elimination of the homozygote *rr* due to an increasingly inadequate transfer of the $\rho$ cytoplasm.

In the wild population the homozygous recessive should occur with up to one quarter of the frequency of the heterozygotes. The latter are common in the wild population but there is no evidence that homozygous recessive plants exist in nature; *i.e.* the gene *r* and its analogues may be regarded as lethals which are only viable in the homozygous condition in $\rho$ cytoplasm.

Two other interesting features of this maternal inheritance may be noted: (1) the absence of resistant plants in the outcrosses of susceptible plants derived from the resistant material and (2) its transmission to succeeding generations through the male side.

The first of these features may be illustrated by the following data: 39 heterozygous susceptible plants were outcrossed to unrelated susceptibles, including the susceptible parents of families 108-112, 136-138, 145-148, and no resistant progeny were obtained. In spite of this, normal inheritance was demonstrated in subsequent generations which again suggests that the cytoplasmic factor was not present in sufficient quantity.

A further slight decrease in the amount of cytoplasmic material transferred would be sufficient to account for the total absence of resistant plants in these crosses. Thus in the resistant outcrosses, excluding the original outcross against which no comparable susceptible outcrosses were available for comparison, 26 resistant plants were found in a population of 1996, so that in a total population of 1210 plants in the susceptible outcrosses only 5·6 would be resistant.

The second feature of the maternal effect noted above, *i.e.* that it is transmitted to a succeeding generation through the male side, has already been demonstrated in table 1 in which the normal behaviour of the gene *r* is shown. In this table paternal and maternal derivations are not distinguished since no differences were observed; about two-thirds of this material was paternally descended from resistant plants. Also, in table 2 one parent of each of the families 87, 88, 89, 95, 96, 97 was paternally derived from the original resistant plant and no differences were noted between reciprocals. These facts suggest that the cytoplasmic material $\rho$ is either produced *de novo* by *rr* or that enough is transmitted to the embryo via the pollen tube to allow normal expression of *rr* to take place in the next generation. The first alternative is incompatable with the maternal mode of inheritance described, but the second is in accord with the facts (see text-fig. 2). Whether the maternal factor is a self-reproducing cytoplasmic entity or is under nuclear control, its important function evidently takes place in the embryo sac nurturing the homozygous recessive.

### (e) *The resistant plant, physiological correlations*

*Viability and seed size.*—Resistant families have smaller seeds than susceptible families; average weights being 1·4 mgm. and 2·4 mgm. respectively. This was most noticeable in family 86 a cross between

resistant daughters of the original resistant plant, in which all seed was brown and withered, and it is here also that fertility was most impaired (3·1 per cent. of pollinated florets setting seed compared with 40 per cent. in the remaining resistant families). In later generations the mean seed weight per family varied from 0·49 mgm. to 2·4 mgm.

In the resistant families which gave anomalous segregation of susceptible plants (families 99, 100, 105) the seed size was always smaller where the more recently outcrossed plant was the male parent. This suggests that the small seed size is not simply a result of development in the embryo sac of a resistant plant of low vitality but is an expression of the gene *r*; a suggestion also borne out by the larger seed size of progeny of outcrossed resistant plants.

The connection between seed size and resistance in segregating families was examined in a cross between heterozygous plants (family 15) in which large variation in seed size obtained. The material was divided visually into five size classes each containing about the same number of seeds. Of 188 plants 54 were resistant (the 1 : 3 expectation was 47). Resistant plants occurred in decreasing proportion in the larger size classes; the numbers of susceptible and resistant plants in the five classes in increasing size order were respectively 11 : 10, 25 : 10, 26 : 11, 34 : 14, and 38 : 9. This result confirms the suggestion that the factor *r* as well as the cytoplasmic component decreases seed size.

There is thus a connection between seed size and resistance, and an experiment was next undertaken to determine whether artificial shortening of the period of embryo growth within the embryo sac had any effect on the inheritance; it might decrease the proportion of the resistant plants if the cytoplasmic factor is a substance which directly affects resistance.

The experiment was performed using a cross between susceptible plants (family 22). Flower heads were cut prematurely at varying numbers of days after pollination. No seed was obtained by premature harvest earlier than 5 days after pollination and the few seeds obtained (15 seeds from 60 florets pollinated) with so short a period of development on the mother plant were not viable. It is interesting to note that this minimum period of preharvest growth of 5 days for the later completion of embryo formation is the same as that noted for barley (Harlan and Pope, 1922) and rye (Nutman, 1943). The results are summarised in table 7; there is no effect of premature harvest on the proportions of resistant plants.

The cytoplasmic factor is therefore independent of the length of time of embryo development on the mother plant, and the viability of the resistant plant is not affected by premature harvest.

*The mature plant.*—No striking abnormality of top or root, other than the absence of nodules, was noted in the original resistant plant. Of its 17 resistant progeny, 12 survived transplanting into soil and a complete record of their appearance was kept. They differed notably

from susceptible plants in dwarf habit, hairy leaves, leaf chlorosis, and in showing floral abnormalities or in failing entirely to flower.

TABLE 7

*Constant proportion of resistant plants in family 22 after normal and premature harvest*

| Period of pre-harvest growth in days | 5 | 7 | 10 | 16 | 20 | 24 | 32 | 40 |
|---|---|---|---|---|---|---|---|---|
| Mean seed weight in mgm. . | *0·15* | *0·12* | *0·88* | *1·50* | *1·03* | *2·14* | *2·45* | *2·57* |
| *sus : res* . . . . | not viable | not viable | 6 : 2 | 18 : 7 | 7 : 3 | 16 : 8 | 18 : 3 | 21 : 2 |

Three produced no upright stems and remained small, three produced short upright stems crowned by a circlet of bracts which soon withered. Six of the resistant plants flowered ; one bore white flowers and in the remainder anthocyanin was poorly developed.

In one the flower head was strikingly abnormal, the inflorescence consisting of a cylindrical head of very pale pink florets with normal calyx and reduced corolla parts. In the mature flower the standard was incurved and the wings and keel were open exposing the stigma and stamens which protruded from the flower owing to the suppression of the corolla tube. This aberration later appeared among susceptible derivatives and is possibly due to a single independent factor ; it is figured in plate 3 (*a*).

In later generations floral and other abnormalities were not so frequent ; the leaves remained chlorotic but the plants were less dwarf (see plate I (*b*)). Flowering occurred in most of the families raised to the flowering stage though the flower colour was generally pale and the amount of flowering scanty.

*Site of resistance : grafting experiments.*—So far as root growth and branching are concerned resistant plants were normal (see plate I (*a*)). They also responded normally to bacterial secretions in showing root-hair deformations. Large numbers of hairs of both resistant and susceptible plants were examined and the proportions of straight, bent, and characteristically curled hairs were the same on each. Roots were also frequently examined for infection threads and none were found. Resistance is therefore absolute, the bacteria failing to gain entry into the host's tissues. It should be noted in this connection that deformation but no penetration of root hairs has been observed with " avirulent strains " (Chen, 1938) and with nodule bacteria inoculated to plants of different cross-inoculation groups (McCoy, 1932).

Attempts were made to graft together resistant and susceptible plants, with two objects in view : (1) to determine whether resistant or susceptible plants owed their characters to specific substances capable of being translocated across a graft union and (2) to transmit the cytoplasmic effect as such in heredity.

The second object could of course be achieved only by extensive breeding from grafted plants; and had to be abandoned as only two grafts united of the large number tried.

These are figured in plate II, (*a*) and (*b*). The graft "*a*" (an approach graft) consists of a heterozygous susceptible top grafted on to a resistant root, and the graft "*b*" (an inarched graft) of a resistant top on a susceptible heterozygous sister root. It will be observed that the resistant root remained unaffected by the presence of a susceptible top and *vice versa*.

Resistance is therefore not due to the production of an inhibitory substance in the top of the plant which is translocated to the root nor is susceptibility (*vis-à-vis* resistance) due to a nodule forming substance produced in the top of the plant and translocated to the root.

The plants figured were replanted and adventitious roots were allowed to develop from above the graft unions, nodules formed only on the adventitious roots developing from the susceptible top. This result suggests that mobile nodule inhibiting or promoting substances are not produced in the root though these experiments do not of course throw any light on the production of either antagonistic or nodule promoting substances which remain *in situ* in the root.

## 4. THE INTERRELATIONSHIPS OF GENE *r* AND OTHER FACTORS

A certain amount of incidental data is available with respect to the interrelationships of *r* with the sterility allelomorphs S, a previously described ineffectiveness factor *i*, and factors determining nodule number and earliness of nodulation. The two latter characters are relevant to the nature of resistance and are conveniently considered at this stage.

### (*a*) *Sterility allelomorphs*

Linkage of the gene *r* with the sterility alleles S would be shown by marked heterogeneity in for example families 9 to 37 (table 1). These comprise four groups of sib crosses of 4 to 8 families each, and 7 independent families. Only 2 families, one independent, give ratios which depart widely from expectation; the remainder are homogenous ($\chi^2_{(26)}$ for heterogeneity $= 17{\cdot}6$; $P = 0{\cdot}9$) showing that the genes *r* and S are not linked.

### (*b*) *Stage of primary nodulation*

The stage of development (represented by the number of leaves, expanded on the main axis, excluding cotyledons) at which nodule formation begins has been studied in the experimental stocks. Wide variation occurs in this character in the wild population, and in selected early lines and selected late lines, and in the intermediate lines obtained on intercrossing, suggesting that a number of factors are concerned in the early and late habits of nodule formation.

The primary nodulation habit is illustrated among resistant line descendants in table 8. The segregation of resistance and the leaf

TABLE 8

*Independent inheritance of resistance and early or late nodulating habit*

| Family nos. | Resistance | | | Primary nodulating habit E = Early nodulation L = Late nodulation | | |
|---|---|---|---|---|---|---|
| | Type of cross | No. of resistant plants | | | | |
| | | Obs. | Exp. | Type of cross | Number of plants | Mean progeny leaf number at infection |
| 109 * | *rr* × *R* | 4 | ? | ? × E | 130 | 2·32 |
| 115 † | (outcross) | 3 | ? | ? × L | 92 | 2·72 |
| 167 | *Rr* × *R* | 0 | 0 | E × E | 32 | 1·92 |
| 168 | (outcross) | 0 | 0 | E × L | 25 | 2·14 |
| 169 | | 0 | 0 | L × L | 50 | 3·14 |
| 3, 4 | *rr* × *Rr* | 32 | 39 | ? × E | 35 | 3·10 |
| 1, 2 | | 43 | 35 | ? × L | 28 | 4·00 |
| 28 | *Rr* × *Rr* | 3 | 5 | E × E | 17 | 2·91 |
| 15 ‡ | | 74 | 78·25 | E × L | 116 | 3·13 |
| 19 § | | 34 | 21·75 | L × L | 68 | 3·40 |

* 109, also 110, 125, 128, 140. † 115, also 130, 137, 146, 152. ‡ 15, also 18, 27, 29, 30. § 19, also 20, 21, 26.

number at primary nodulation is indicated. Among the susceptible progeny of the various crosses the parental types are simply classified as, either early (E), or late (L), in nodulating habit, *i.e* as nodulating with less than 2½ or more than 3 leaves respectively.

These results show that the progeny of outcrossed resistant plants (families 109, etc., and 115, etc.) are partly dependent in nodulating habit on the susceptible parent. Resistant plants appear therefore to vary in factors affecting earliness in nodulation. This is confirmed in the remaining data in the table; within each type of cross the proportion of resistant plants is not significantly greater among plants of late habit than among other types. The genetic factors which determine the time at which primary infection of the root takes place, thus segregate independently of the gene *r*.

### (c) *Number of nodules*

The number of nodules which develop on the roots of clover are genetically determined. The crossing of contrasting types leads to intermediate types in $F_1$, as was noted in the previous quantitative character. Since the number of nodules also depends on conditions of growth (duration, bacterial strain inoculated and the volume of

the root medium) only an approximate classification can be made of parental types selected at different times, and the results of different experiments cannot be so well collated. Accordingly the inheritance of density of nodule formation and resistance in two separate experiments is shown in table 9 with for comparison (experiment 1) the

TABLE 9

*Independent inheritance of resistance and of factors affecting the numbers of Nodules formed on the root*

| Family no. | Resistance | | | No. of nodules | | Rank correlation Spearman's $\rho$ |
|---|---|---|---|---|---|---|
| | Type of cross | No. of resistant plants | | Parental nodule no. | Progeny nodule no. (mean) | |
| | | Obs. | Exp. | | | |
| Experiment 1 | | | | | | |
| | $R \times R$ (unrelated) | ... | ... | 6×20 | *18·7* | |
| | ,, | ... | ... | 62× 6 | *21·2* | |
| | ,, | ... | ... | 89× 6 | *24·6* | |
| | ,, | ... | ... | 89×62 | *40·7* | |
| Experiment 2 | | | | | | |
| 116 | $rr \times R$ | 0 | ? | 0× 6 | *16·2* | 0·943 |
| 123 | $rr \times R$ (outcross) | 0 | ? | 0×10 | *20·1* | (P = 0·999) |
| 110 | ,, | 1 | ? | 0×15 | *18·9* | |
| 114 | ,, | 0 | ? | 0×16 | *27·2* | |
| 117 | ,, | 0 | ? | 0×20 | *29·7* | |
| 112 | ,, | 1 | ? | 0×62 | *34·7* | |
| Experiment 3 | | | | | | |
| 31 | $Rr \times Rr$ | 35 | 31·0 | 17×17 | *19·7* | |
| 37 | ,, | 3 | 14·25 | 23×17 | *15·6* | 0·714 |
| 33 | ,, | 24 | 18·25 | 20×21 | *19·4* | (P = 0·967) |
| 35 | ,, | 13 | 16·25 | 23×23 | *17·3* | |
| 36 | ,, | 28 | 27·5 | 35×51 | *32·0* | |
| 34 | ,, | 10 | 7·75 | 38×51 | *43·4* | |
| 32 | ,, | 12 | 14·25 | 20×74 | *32·8* | |

number of nodules formed in crosses between some of the susceptible plants used in the original outcrosses of resistant plants.

Within each experiment the families have been arranged in order according to the sum of the parental nodule numbers, and Spearman's rank correlation test (Kendal, 1947) has been applied to the mean progeny nodule numbers. In each experiment the correlation between parental and progeny number is highly significant.

Although the progeny of resistant outcrosses (experiment 2) broadly reflect their susceptible parents in this character, the range of difference is less than in experiment 1, suggesting that the genotypes of the resistant plants correspond to an intermediate number of nodules. These results show that resistance does not correspond genetically to an extreme form of sparse nodulation.

### (*d*) *Independent segregation of the factors* I *and* r

Ineffectiveness in nitrogen fixation—which is shown under the conditions of growth employed by absence of dry weight increase—may be due either to nodulation by an ineffective strain of bacteria or to genetic influences in the host plant (Nutman, 1946). In the latter case a simple factor *i* has been shown to influence the symbiosis in inducing an ineffective response with the normally effective strain of bacteria used throughout this investigation. This factor has been found both within and outside the resistant lines and in table 10

TABLE 10

*The independent segregation of the factors* i *and* r

(I, effective. ii, ineffective.)

| Family no. | Cross type | | Effectiveness | | | $\chi^2$ |
|---|---|---|---|---|---|---|
| | Resistance | Effectivity | Observed | | Deviation from expectation | |
| | | | I | ii | | |
| 4, 5 | *rr* × *Rr* | ? × i | 15 | 17 | +1·0 | 0·125 |
| 6, 7 | ,, | ? × Ii | 17 | 5 | +0·5 | 0·061 |
| 17, 20 | *Rr* × *Rr* | ii × Ii | 12 | 16 | +2·0 | 0·571 |
| 18 | ,, | Ii × Ii | 16 | 7 | +1·25 | 0·362 |

its independent inheritance is demonstrated. The families 4, 5, 6 and 7 in the table have a common resistant parent which is evidently heterozygous for the ineffectivity factor. The progeny of families 6 and 7 provide the parent plants for the families 17, 18 and 20. In the table the observed numbers of ineffective (*ii*) and effective (I) plants are shown; an excess of ineffective plants is regarded as a positive deviation.

It has already been shown (table 1) that resistant plants segregate normally in these families, and it is clear from the goodness to fit of the segregations for the gene *i* that its inheritance is independent of *r*. The total positive deviation for ineffectivity is probably due to inbreeding.

## 5. GENETIC ABNORMALITIES IN THE RESISTANT LINES AND DERIVATIVES

The frequent occurrence of abnormalities in red clover is of some importance in view of the above suggestion that the maternal effect may allow the development of plants with otherwise lethal genes in the homozygous condition, or alternatively that the cytoplasmic component augments the action of genes normally having small effects.

Complete records have been kept of the very great variety of observed aberrations and for convenience they have been classified into two main categories: (1) those which appear, often on slender evidence, to be inherited as simple recessives, and (2) those which have no clear mode of inheritance. The second category comprise abnormal germination, dwarfing, chlorosis, variegation, cotyledon deformation, etc.

### (*a*) *Simple recessives*

The distributions of six recessive factors in the resistant line material and its derivatives (about 200 families), and in about the same number of unrelated families are listed in table 11. The following are brief descriptions of these forms :—

*Albino w.*—Williams (1939) distinguished 8 albino recessive factors. Some pure white, others cream or golden seedlings ; similar distinctions found in present material.

TABLE 11

*The distribution of simply inherited abnormalities within and outside the resistant lines and derivatives*

| Character | Family no. | Segregation | | | No. of non-segregating related families |
|---|---|---|---|---|---|
| | | Normal | Mutant | $\chi^2$ | |
| *Albino w* . . | 23, 79-82, 177, 193 (7) | 173 | 48 | *20·84* | 5 |
| | 50, 62, 63, 74, 84 (5) | 121 | 27 | *12·08* | 10 |
| | 34 (1) | 33 | 8 | *0·66* | 2 |
| | ... (4) | 67 | 20 | *2·68* | 4 |
| | ... (2) | 31 | 13 | *2·49* | 1 |
| *Blue-green* . | 26 (1) | 29 | 9 | *0·04* | 4 |
| *Dwarf bgd* . | ... (1) | 13 | 3 | *0·33* | 1 |
| *Crimped leaf cl* . | ... (3) | 28 | 9 | *0·33* | 2 |
| *Pink flush pf* . | 180, 181 (2) | 31 | 13 | *1·85* | 0 |
| *Stump root sr* . | ... (1) | 21 | 8 | *0·10* | 0 |
| *Raspberry head rh* | progeny of original outcrosses | ... | 1 | ... | ... |
| | 102, 104 (2) | 9 | 4 | *0·24* | (?) |

Five cases of single factor albinism encountered ; three among descendants of the resistant plant, two elsewhere.

Overall ratios support a single factor basis, but great variation between families occurred in the first two instances : linkage with the S factor may be involved as demonstrated for factor $w_1$ by Williams (1939).

*Blue-green dwarf, bgd.*—Cotyledon size and colour normal, the first leaf blade much reduced, generally irregularly triangular in shape, dark in colour. Succeeding leaflets remain infolded, hairier than normal leaves. No effective growth is made, a plant becoming smaller as each withered leaf is replaced by a smaller succeeding one. Semi-lethal, no flowers are produced although a single plant transferred to soil survived for 9 months. Resistant and susceptible blue-green dwarfs occur in family 26. Both types of seedlings with an older

plant are illustrated in plate III, (*b*) and (*c*). The character is due to reduction in the cell size without reduction in plastic number or size. Affected cells have denser protoplasmic contents, only vacuolated along mid-rib of leaf.

Outside the resistant material blue-green dwarfs appeared among $F_2$ plants of a cross between unrelated susceptible plants one of which was, however, related to family 26. The original resistant plant was therefore probably not heterozygous for this character.

*Crimped leaf, cl.*—Morphologically related to blue-green dwarf as it concerns abnormal leaflet infolding and is progressive. The first four leaves normal but leaflets of following leaves do not unfold. Infolding at first restricted to the tip of the leaflet, extends in later leaves to include the whole length of mid-rib, and occasionally the whole of the breadth of the leaflet. Probably inherited as a simple recessive, does not occur among resistant plants and their descendants.

*Raspberry head, rh.*—Described above in the description of resistant plants. The inflorescence has compact appearance resembling a large unripe raspberry (see plate III (*a*)). First appeared in the progeny of the original outcrossed resistant plant which was therefore heterozygous for this character, confirmed by its later appearance among descendants of other outcrosses.

The data available is very sparse ; a simple factor probably concerned. Original raspberry headed plant resistant ; of the four later ones two resistant and two susceptible.

*Pink flush, pf.*—A bright pink colouration of hypocotyl and upper part of primary root, usually extending into and intensified in first nodules ; intensity of colouration varies. Appeared in two families with one common parent. Improbable that the original resistant plant was heterozygous for this factor.

This recessive is of some interest in view of the recent work on hæmoglobin in nodules (Keilin and Wang, 1945 ; Virtanen, 1945). Section of pink-flush nodules show that the colour is developed in the nodule rind whereas hæmoglobin is confined to the bacterial tissue.

*Stump root, sr.*—Very slow growth in length of roots, few branches, a dark brown-green colouration of surface of root. Nodules form sparsely, tend to grow to large size as shown in plate III, (*d*). Tops are at first normal, become dwarfed and withered, probably due to interruption in uptake of nutrients. Occurs only in one family, unrelated to the resistant lines.

The distribution of these six recessives in the experimental stocks shows that simply inherited abnormalities do not appear to be more or less frequent than usual among the descendants of the original resistant plant.

### (*b*) *Genetically indeterminate forms*

The various types of abnormality of indeterminate inheritance in material of different origin are not separately listed in tables 12 and 13,

instead only the percentage germination and the percentage of abnormal plants are shown. In table 12 data for seed set is also included.

TABLE 12

*Seedling abnormalities in red clover (5671 seeds)*

| Family type ♀ ♂ | Percentage set of seed | Total no. of seeds sown | Percentage germination | Percentage abnormal seedlings |
|---|---|---|---|---|
| *rr* × *R* (seg) . . . | 44·5 | 567 | 85·4 | 19·6 |
| recip. . . . . | 60·7 | 358 | 85·2 | 2·3 |
| *rr* × *R* (non-seg) . . | 38·7 | 580 | 82·8 | 19·4 |
| recip. . . . . | 48·3 | 619 | 93·2 | 4·3 |
| *Rr* × *R* . . . . | 59·0 | 273 | 83·9 | 9·2 |
| recip. . . . . | 53·6 | 232 | 90·9 | 8·5 |
| *Rr* × *Rr* (and recip.) . | Normal i.e. | 334 | 82·0 | 21·2 |
| *rr* × *Rr* (and recip.) . | 50-60 | 511 | 89·3 | 17·8 |
| Original selection M.R.C. | ... | 1709 | 89·0 | 14·0 |
| Backcross . . . | ... | 228 | 94·3 | 14·0 |
| $F_2$ . . . . | ... | 260 | 78·0 | 30·7 |

The first group of entries refers to the outcrosses of resistant plants and it is clear that abnormalities occur more frequently in outcrosses involving resistant plants as maternal parents than in reciprocals, a difference is apparent whether or not resistant plants segregate in the families concerned.

The two following rows of entries show that this maternal effect is not evident in the next generation outcrosses, a relatively low incidence of abnormalities paralleling the absence of resistant plants. The next types of cross shown indicate that the proportion of abnormalities increase in the next generation and also in the progeny of backcrosses to resistant plants, due no doubt to recombination. In the latter type of cross no consistent maternal influence was noted.

These results may be compared in the lower part of the table with data obtained from outside the resistant line. The average percentage of abnormality in the outcrosses of resistant mother plants and reciprocals about equals that of original crosses outside the resistant line material (12 and 14 per cent. respectively). The reciprocals of outcrosses of resistant mother plants thus yield a higher proportion of normal plants than any other type of cross. On inbreeding, about the same number of abnormalities reappear outside and within the resistant line though the number of plants for comparison here is not large.

These results suggest that the resistant line is not intrinsically richer in genetic factors leading to abnormal development, but rather that abnormalities are more frequent at the first interaction of certain unspecified genotypes with the cytoplasm of a resistant plant. In this connection it may be significant that the majority of the

abnormalities listed are seedling characters; later development being mostly normal. This holds for most forms of dwarfing and for variegation which is largely confined to the cotyledons and is rarely systemic. Leaf abnormalities are generally confined to the first and second leaves, the most frequent being the occurrence of two opposite and decussate unifoliate "first" leaves instead of a single unifoliate first leaf. The principal root abnormality is the abortion of the primary radicle.

Throughout this phenomenon there appears therefore to be a disorganising maternal influence which becomes less potent as the plant develops; only in the case of the resistant character, which is a mature plant character, is the maternal influence persistent and perpetuated. This confirms the suggestion already made that the maternal influence is itself a product of the resistant factor *r*.

Outside the resistant line considerable and quite significant differences between reciprocals are not infrequent. The distinction, however, between the occurrence of maternal effects in the two kinds of material is that in the resistant outcrosses the differences between reciprocals are consistently in one direction. It is possible that if an hereditary character were available to sort out the maternal effects outside the resistant line a similar disposition of the abnormalities would be found.

In table 13 the seedling abnormalities of resistant families are shown.

TABLE 13

*Distribution of seedling abnormalities in the resistant line*

| Cross type $r \times r$ family no. (see table 2) | No. of seeds sown | Percentage germination | Percentage abnormal seedlings |
|---|---|---|---|
| 86 | 42 | 45·2 | 100·0 |
| 87, 88, 90, 91 | 228 | 63·2 | 39·6 |
| 96 | 47 | 55·3 | 23·1 |
| 97 | 89 | ... | 6·6 |
| 92, 94, 95, 98 | 270 | 82·3 | 23·5 |
| 94, 101, 102, 103 | 164 | 67·5 | 43·2 |
| 105-108 | 121 | 90·1 | 43·1 |
| 99, 100, 105 One parent outcrossed in previous generation | 156 | 84·7 | 12·0 |

With the exception of the last group (families 99, 100, 105), the families are arranged according to the closeness of their relation to the original resistant plant; the seventh group (families 105-108) being four to five generations removed from the original resistant plant.

Progeny of the crosses between resistant daughter plants of the original resistant plant (family 86) show a maximum number of abnormalities, which was reflected in both the high incidence of

dwarfing, abnormal germination, chlorosis, etc., and also in a high proportion of non-viable seed.

In later generations some of the abnormalities have been eliminated and the proportion of normal plants tends to increase and appears to be of the same magnitude as in comparatively inbred material outside the resistant line. Although the material is descended from a single parental resistant plant, a number of different susceptible plants entered into the ancestry in outcrosses. For example the family 97 in the table is derived from four different susceptible plants and as a result the effects of inbreeding are not so evident. In the last three families listed in the table recent outcrossing to unrelated susceptible plants has taken place and the normal type of growth has been restored.

The large numbers of abnormalities which appear in resistant crosses and in resistant outcrosses do not appear to be open to interpretation along orthodox genetic lines.

The alternative physiological explanation, *i.e.* that they simply reflect the impaired nutrition of the embryo is genetically insufficient because it fails to account for the correlation between the distribution of these abnormalities in this material and the maternal component in the inheritance of resistance itself. This aspect has accordingly been dealt with in some detail and its relation to other cases of extra-chromosomal inheritance will be more fully discussed below.

## 6. GENETICAL ASPECTS OF RESISTANCE

The principal genetic interest in resistance lies in the cytoplasmic gene interactions which have been disclosed, and in fig. 1 simplified pedigrees illustrate the essential features of the inheritance.

The original hypothesis was that the inheritance of resistance might be explained by the simple interaction of a recessive gene with a cytoplasmic component. This has proved not to account for all the facts. The results at variance with this simple hypothesis are of two kinds : (i) the partial or complete elimination of resistant plants in mixed families of various origin—marked as a crossed circle in fig. 1 ; and (ii) the intrusion of susceptibles in the resistant lines.

(i) The degree of elimination of resistant homozygotes in different crosses is shown in table 14. In the original outcross probably no elimination occurred but in each succeeding generation of outcross of resistant mother plants with unrelated susceptibles of type B the elimination becomes more marked ; it is everywhere complete in the reciprocal crosses and in crosses among susceptibles of type B.

In these outcrosses this elimination is correlated with the maternal mode of inheritance and has been ascribed to the absence of $\rho$ cytoplasm in the embryo-sac of the susceptible parent. The depression of seed set in the families in which elimination occurs only partly accounts for the deficiency of resistant plants and germination is

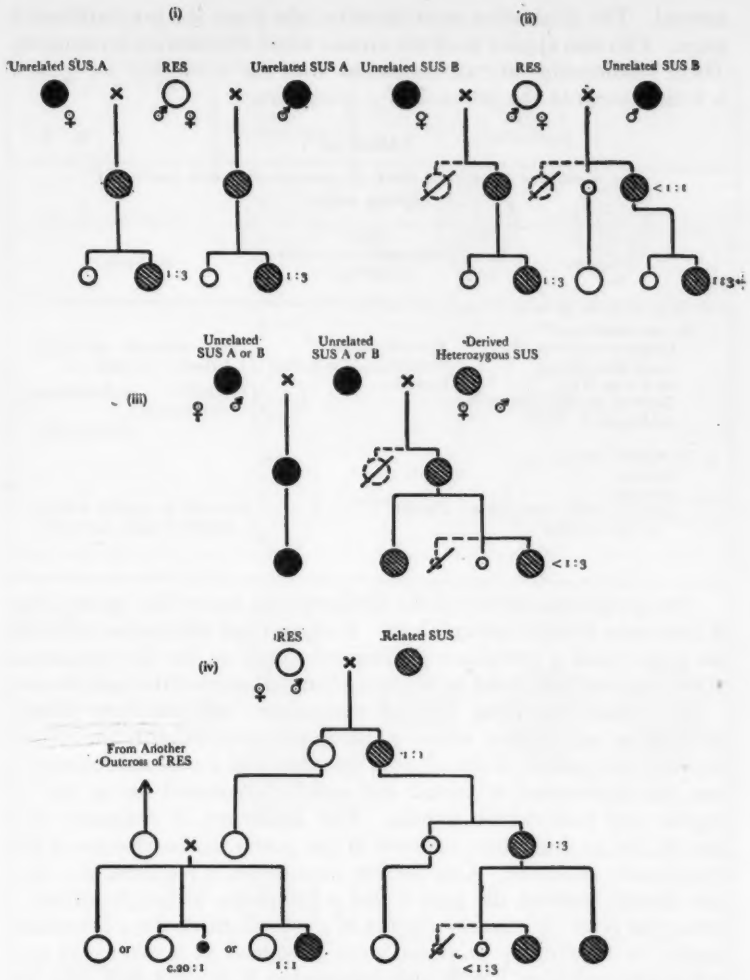


FIG. 1.—Abridged pedigrees illustrating the inheritance of resistance (lines of descent from single type plants represent sib matings). Unrelated susceptible plants shown by black circles and susceptibles related to resistants by hatched circles ; resistant plants shown by open circles ; relative proportion indicated by size of circle. Elimination of resistant plants indicated by crossed out circles. (i) and (ii) show reciprocal crosses of *res* $F_1$ plants with unrelated susceptibles of types A and B. With type A, no resistants appear in progeny ; with type B, resistant plants appear as less than half the daughter plants of resistant mothers. In remaining crosses a normal 1 : 3 ratio occurs of the resistant plants breed true. (iii) shows the pure breeding of unrelated susceptibles and the elimination of resistants in crosses of derived susceptibles with unrelated susceptibles. (iv) shows segregation following the crossing of related resistant and susceptible plants. The deficiency of resistant plants in mixed families with an intercalary susceptible generation, and the appearance of anomalous susceptibles in some resistant lines is also indicated.

normal. The elimination must therefore take place at a pre-fertilisation stage. This also applies to all the crosses where elimination is complete. These relationships are all consonant with the view that the gene *r* is lethal except in the presence of ρ cytoplasm.

TABLE 14

*The elimination of resistant plants in genotypically mixed families of differing origin*

| Family | Elimination of recessive homozygotes | Remarks |
|---|---|---|
| 1. In outcrosses :— | | |
| Original *res* ♀ × *sus* B ♂ | Probably none | Partly detected as zygotic lethals ; see table 12 |
| Later generations | Progressive elimination | |
| *res* ♂ × *sus* B ♀ | Complete | Probably prefertilisation elimination |
| Derived *sus* ♀ or ♂ × *sus* B | ,, | |
| *sus* B × *sus* B | ,, | |
| 2. In related lines :— | | |
| *res* × *sus* | None | ... |
| *sus* × *sus* | ,, | ... |
| *sus* × *sus* (with intercalary *sus* generation) | Partial | Detected as zygotic and post zygotic lethals (see text) |

The progressive nature of the elimination in succeeding generations of outcrosses remains unexplained. It suggests an adaptation between the gene *r* and ρ cytoplasm leading eventually to the disappearance of the maternal effect and an apparent simplification of the inheritance.

In crosses involving related susceptible and resistant plants, elimination only occurs where a susceptible generation is interposed between the parents of the cross in question and a resistant ancestor ; here the elimination is partial and can be accounted for as due to zygotic and post-zygotic lethals. This deficiency of resistants may also be due to the lethal character of the gene *r* in the absence of the cytoplasmic substance. One further assumption is required, *viz.* that the relation between the gene *r* and ρ cytoplasm is complementary ; just as the gene *r* is lethal in absence of ρ cytoplasm, so the ρ cytoplasm cannot be indefinitely produced or is produced at a decreased rate in the absence of *rr*. With this assumption it is clear that with an intercalary susceptible generation a dilution of the ρ cytoplasm could occur and this would lead to the elimination of resistants. Here elimination occurs at a later stage than in outcrosses, *i.e.* by zygotic and post-zygotic lethals.

(ii) In contrast to the above, an explanation of the occurrence of susceptible plants in crosses between resistant parents has already been offered in terms of additional genetic factors and is supported by breeding experiments. Here resistant factors are always present and the cytoplasmic component may be supposed to be under constant production.

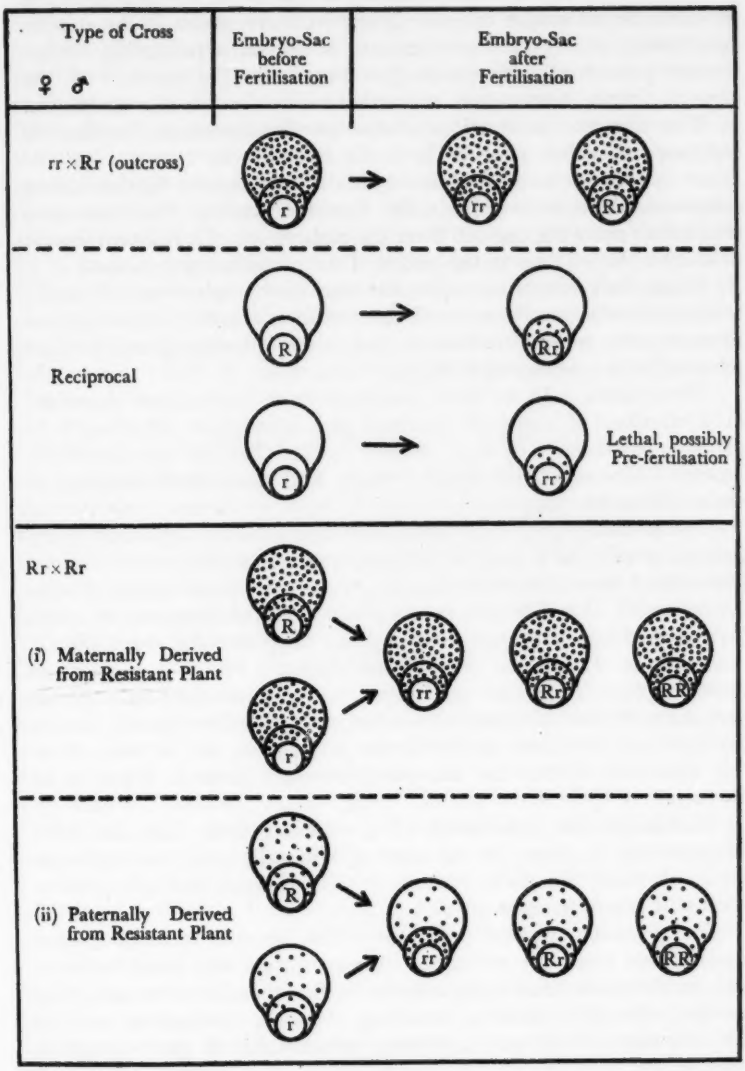


FIG. 2.—An explanation of the maternal inheritance in terms of the location of the cytoplasmic resistance material $\rho$, which is represented in the diagrams by heavy stippling when transmitted through the egg and light stippling when transmitted through the pollen.

It is therefore suggested that the original hypothesis be modified as follows : (1) the resistant condition is due to the interaction of one of a number of simple recessive genes with cytoplasm of the specific constitution $\rho$ ; (2) the $\rho$ cytoplasm is not self-reproducing or has limited powers of self-reproduction but is itself a product of the gene *r* ; (3) the homozygote *rr* is lethal in the absence of $\rho$ cytoplasm.

The effective production of the specific cytoplasm leading to resistance therefore requires both the homozygous recessive genetic factor and the presence of a quantity of this substance in the developing *embryo-sac* which is to contain the recessive zygote. This substance may either enter the egg cell from the embryo-sac of a resistant female or else be carried over in the pollen of the preceding generation.

Using the symbols $\rho$ to represent resistant cytoplasm and R and *r* to designate the genes concerned, the condition of embryo development in segregating resistant outcrosses and in the following generation are schematically represented in fig. 2.

The scheme calls to mind gene-cytoplasm interactions described in a number of cases of maternal non-Mendelian inheritance in plants ; particularly in male sterility in flax (Bateson and Gairdner, 1921 ; Chittenden and Pellew, 1927) and chlorophyll striping in maize (Rhoades, 1943).

The intussusception of a genetically determined cytoplasmic entity (plasma gene) which may be self perpetuating, or at any rate can be transmitted from parent to offspring, is also postulated among micro-organisms in the inheritance of certain adaptive enzymes in yeast (Winge and Lausten, 1940 ; Lindegren, 1945) and for the " killer " character in *Paramecium* (Sonneborn, 1946). The $\rho$ cytoplasm of resistant plants resembles the kappa (K) substance in *Paramecium* in that some of the substance must be present before genetic factors can have any influence on its further production, but it differs from this substance in that the associated recessive factor is lethal in its absence.

Non-Mendelian inheritance of a different order has also been demonstrated in plants by the work of Michaelis (1937) on *Epilobium* species hybrids, by Sirks (1931) on *Vicia* species hybrids and by Wettstein (1928) in mosses.

The present researches may form a link between these two types. On the one hand the resistance-type cytoplasm has specific effects, and on the other hand it contributes to the appearance of abnormal seedling characters such as dwarfing, chlorosis, variegation, etc., in the outcrosses of resistant ♀ plants ; possibly due to gene-cytoplasm interactions.

Further, from the extensive survey outside the resistant line and its derivatives, it has been shown that examples of non-Mendelian inheritance involving similar kinds of seedling abnormality are fairly widespread in the wild population of red clover. That these sporadic cases may be explained in similar terms is suggested by their occurrence

with the same relative frequency as within the outcrosses of resistant plants irrespective of the direction of the cross. The fact that abnormalities occur much more frequently in the outcrosses of resistant ♀ plants than in the reciprocal crosses, which show the highest proportion of normal plants, again suggests a correspondence between these maternal effects inside and outside the resistant line material. That these reciprocal differences are not generally remarked, and their maternal mode of inheritance studied, is possibly because the genetic factors responsible for the multiplication of the cytoplasmic component have not been detected. Within the resistant line outcrosses the maternal effects can be distinguished because they are controlled by a mutant gene affecting a simple character (as in *Paramecium*). The almost complete elimination of abnormalities in the crosses of *sus* ♀×*res* ♂ also suggests, either that there are few mutant loci able to affect the cytoplasm in this way, or that different mutations may have similar effects on the gene-cytoplasm interaction.

## 7. RESISTANCE IN LEGUME SYMBIOSIS

In symbiosis resistance is a relative term, and its use is not always appropriate. Gaumann (1946) has shown that a similar problem of definition is met with in pathology, particularly in for example rust fungi infections, and he suggests that total absence of infection is not properly described as resistance since no detectable response of the host plant is invoked. He describes such conditions as " axeny "—inhospitality. This point of view is also more appropriate when the inheritance of other factors affecting the symbiosis is considered specially in the determination of the number of infections which occur on the roots of susceptible plants ; differing degrees of hospitality rather susceptibility being shown. Bacterial virulence is probably not involved ; nodule formation seems to be determined largely by host factors (Nutman, 1948, 1949). Recent work with *Medicago sp.* (Aughtry, 1948) shows that heritable factors are also concerned in cross-inoculation group specificity.

On the physiological plane the unusual mode of inheritance is of additional interest. If resistance is due to a cytoplasmic material whose production is under nuclear control, it must be able to reach to the cell wall of the root hair so as to be able to prevent penetration by the bacteria. Grafting experiments have shown that there is no transfer from root to shoot of substance affecting the infection or *vice versa* so that the hypothetical substance must be very local in its movement. This is not surprising in view of the very specialised route of infection normally taken.

Resistance to infection may be due either to the presence of a substance conferring immunity, or to the absence of a substance necessary for infection. On the first supposition, the existence of more than one gene for resistance would suggest the formation of

different substances conferring resistance rather than the production of a single substance by different genetically determined biochemical routes. On the second supposition the different resistance genes may operate by blocking different stages in a chain of biochemical reactions leading to the production of the substance necessary to infection.

In connection with the spread of the nodulating habit in the *Leguminosæ* (or of resistance to infection with a restricted group) a cytoplasmic mode of inheritance controlling infection is of obvious significance especially if it was originally transmissible by infection in nature, *i.e.* by a virus.

These speculations may be useful in suggesting modes of approach to the problems of nodule formation, and closer study of the resistant plants themselves may lead to a better understanding of infection.

## 8. SUMMARY

1. An hereditary resistance in red clover to infection by nodule bacteria is described. Resistant plants are generally less vigorous than susceptibles, more chlorotic and hairier and show floral abnormalities. Root hairs of resistant plants show the characteristic curling in the presence of nodule bacteria but are not penetrated. On grafting no transference of resistance or susceptibility occurs.

2. Resistance is inherited as a simple recessive (*r*) which acts in conjunction with a maternally transmitted component (specific cytoplasm $\rho$).

3. Maternal inheritance occurs only in certain crosses of resistant female plants with unrelated susceptibles (*i.e.* in outcrosses). Such crosses yield in some families (*a*) wholly susceptible progeny, and in other families (*b*) mixed progeny; the reciprocal families being wholly susceptible. The proportion of resistant segregates of (*b*) approach 50 per cent. in the original outcross and decrease with each succeeding outcross generation. Unrelated susceptibles of types (*a*) or (*b*) breed true.

4. A partial elimination of resistant plants occurs in mixed families with an intercalary susceptible generation between the susceptible parent and a resistant ancestor which is accounted for as due to zygotic and post-zygotic lethals. This elimination is assumed to be due to the dilution of the cytoplasmic factor.

5. Resistant plants breed true except in some of the families which derive from more than a single known susceptible outcross ancestor. In such families a variable proportion (up to 50 per cent.) of susceptibles appear; breeding experiments suggest that supplementary factors are concerned.

6. Resistance is assumed to be due to a cytoplasmically located substance the production of which is conditioned by the recessive factor *r*. In the absence of this substance in the developing embryo-sac, *rr* zygotes are normally lethal; it is thus an essential metabolite

and without some of it already being present it cannot be produced by *rr*.

7. The recessive resistance gene (*r*) is not linked with the sterility alleles (*S*), and is inherited independently of factors determining primary nodulating habit (earliness or lateness), the number of nodules formed, or a recessive factor (*i*) for ineffectiveness in nitrogen fixation.

8. Simply inherited morphological abnormalities are not more frequent among the resistant lines and derivatives than in unselected material. The following six simple recessives were identified :— albino (*w*), blue-green dwarf (*bgd*), crimped leaf (*cl*), raspberry head (*rh*), pink flush (*pf*) and stump root (*sr*).

9. Certain genetically indeterminate abnormalities (especially dwarfing, chlorosis and abnormal germination) are more frequent in resistant ♀×susceptible than in the reciprocal crosses independently of the segregation of resistant plants. Similar reciprocal differences also occur sporadically in the wild population at a frequency equal to that in the combined outcrosses of resistant female plants and reciprocals, suggesting a similar mode of inheritance. Elsewhere in the experimental stocks such abnormalities occur with the same frequency as in a comparatively inbred wild population.

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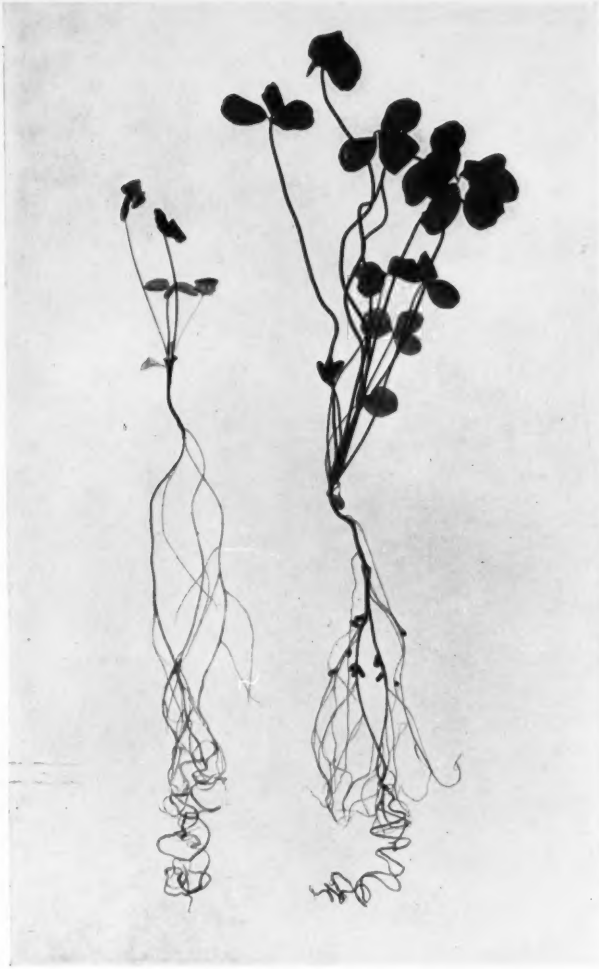
PLATE I.—Resistant and susceptible clover plants



(*a*) Resistant and susceptible clover plants from an agar culture (× 1). (*b*) Three resistant plants (on left) and two susceptible heterozygous plants grown in soil with added nitrogen fertiliser.

PLATE II.—Grafts of resistant and susceptible clover plants

(*a*) Approach graft of heterozygous susceptible top on resistant root.

(*b*) An inarched graft of a resistant top on a susceptible root; note good nodulation (× 1½).

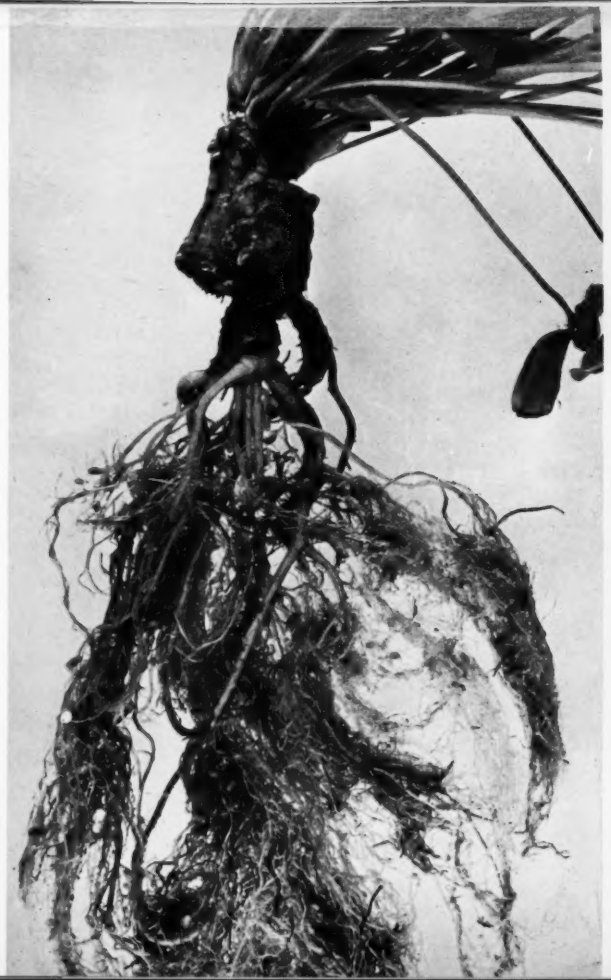


PLATE III.—Simple recessives

(*a*) "Raspberry head," an inflorescence abnormality (×2½).

(*b*) A "blue-green dwarf" plant, nine months old, growing in soil (×¼).

(*d*) Normal and "stump-rooted" plants from an agar culture (×1).

(*c*) Resistant and susceptible blue-green dwarfs from an agar culture (×1).

# A SELF-PROPAGATING STRUCTURAL CHANGE IN *TRITICUM*

## II. The Reproductive Cycle

By O. H. FRANKEL

*Wheat Research Institute, Christchurch, New Zealand*

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### 1. INTRODUCTION

Dicentric chromatids, broken unevenly in a meiotic anaphase, may yield functional gametes with inverted duplications of part or the whole of a chromosome arm, as was shown in maize by McClintock (1938, 1939, 1941) and inferred in wheat in the first paper of the present series (Frankel, 1949). Chiasmata in such duplications, whether within or between partner chromosomes, may—and the majority do—yield bridges and fragments, a process which results in eliminating the duplication (Frankel, *l.c.*). Yet the new bridges are liable in turn to break unevenly, serving as a source of new duplications not necessarily identical with those to which they owe their existence. Thus we find a *chiasma cycle* from generation to generation, commencing with a duplication chiasma in one generation and leading through an unevenly broken bridge to a duplication chiasma in another.

Dicentric chromatids are of widespread occurrence, both in controlled experiments and in natural populations. It is therefore surprising that inverted duplications arising from bridge rupture have not been observed more frequently; for at least in plants, and especially in polyploids, they have a chance of survival.

In a dwarf *compactum* type of wheat, Håkansson (1933) illustrated what is probably a homozygote for an inverted duplication. Loops were seen in metaphase I with a frequency of about 10 per cent.; anaphase I and II were not observed. Derivation and progeny were not recorded.

Richardson (1936) found inverted duplications in *Lilium*. She also illustrated what appears to be a univalent bridge, which may have

originated from chiasma formation in a univalent with a duplicated and inverted segment. Similarly, the likelihood of inverted duplications is suggested where dicentric chromatids are of common occurrence and where there are unusually high frequencies of second anaphase bridges, as in *Tulipa* (Upcott, 1937*a*) and in *Tradescantia* (Bhaduri, 1942). Upcott also found univalent bridges, here, as in *Polygonatum* (Suomalainen, 1947), suggesting an inverted duplication in a univalent.

It must be recognised that whatever the number, the external or the internal relationships of chiasmata, the only structural or genetical change which is brought about by them is the elimination of the duplication. The duplication is perpetuated by non-crossover and normal crossover chromatids, but removed as a fragment when crossing-over leads to bridge formation.

The chiasma cycle preserves the duplication from immediate extinction ; yet each bridge potentially contributes towards the ultimate extinction of the duplication. Not only are there median breaks which fail to yield duplications, but for each duplication chromatid there is a corresponding deficiency chromatid, and some or all of these may be viable. Hence the existence of the duplication is determined by chiasma formation. There is in fact a wider duplication cycle, from the inception of a duplication to its ultimate removal by crossing-over, from the first to the last unevenly broken bridge. The length of this " reproductive cycle " is primarily determined by the chiasma frequency ; where this is high the cycle will be brief.

Is it likely that inverted duplications play a part in the origin of duplications which become part of the stable chromosome complement? Circumstantial evidence comes from " reversed repeats," and, possibly, " doublets " and other associations of small numbers of identical or similar bands in the salivary gland chromosomes of *Drosophila* and *Sciara* (*cf.* recent review by Metz, 1947). The association of a number of chlorophyll genes in the distal sector of the short arm of chromosome 9 (McClintock, 1944, 1946 ; *cf.* also Metz, *loc. cit.*), which is known to yield inverted duplications, is also suggestive ; and so perhaps are the suspected terminal duplications or deletions in *Drosophila ananassæ* (Kikkawa, 1938 ; Dobzhansky and Dreyfus, 1943).

To be incorporated in the stable complement of a species, an inverted duplication, in common with other structural changes, must fulfil three conditions : it must permit survival of the cell and of the organism ; it must have a physiological advantage ; it must become stabilised. It is the last which raises a specific problem in the case of an inverted duplication. Stabilisation is possible only after the elimination of internal crossing-over. This will occur where chiasma formation is inhibited—either through an additional structural change such as translocation or inversion, or by a special genotypic condition such as localisation.

The subject of this paper is the inheritance—and in consequence

the chiasma cycle—of the wheat duplications which have been described previously. Once the cytological types and their frequencies are known, it is possible to predict the duration of the reproductive cycle. This prediction has been tested in a population derived from a known duplication type.

The following abbreviations are used :—

| | |
|---|---|
| ABC | chromosome (bivalent) in which duplications occur. |
| BC | arm of above chromosome in which duplications occur |
| AB | normal arm of above chromosome |
| LD | gamete with a long duplication |
| SD | gamete with a short duplication |
| SD*ef* | gamete with a short deficiency |
| N | gamete without a duplication |
| O | gamete lacking the ABC chromosome. |

## 2. THE TRANSMISSION OF CHROMATIDS RESULTING FROM BRIDGE BREAKAGE

In maize the fate of broken bridge chromatids was followed cytologically by observations in pachytene, and genetically by observations of endosperm and plant characters (McClintock, 1939 and 1941). Wheat chromosomes are not suitable for pachytene study, and no genes are known in the duplication arm. The transmission of broken chromosomes was therefore followed by the cytological identification of zygotes in the progenies of crosses and selfings of the duplication types. They are recorded in table 1.

### (*a*) *Qualitative*

Information on the viability and the nature of the products of bridge breakage is first derived from selfed homozygotes and monosomics for the long duplication, LD/LD and LD/O. In their progenies any D chromosome without the long duplication comes from a broken bridge chromatid. The presence in these families of LD/N, LD/SD, SD/SD, SD/N, N/N and SD/O plants proves (i) that gametes containing chromosomes derived from broken bridge chromatids are functional, and (ii) that bridge breakage produces SD and N gametes.

As noted previously and as might be expected from their origin through chromatid breakage, there is some variation in the size of the short duplications ; but, contrary to the observations by McClintock (1941) in maize, the size varies within narrow limits. The difference between wheat and maize, one may presume, is due to the relative sizes of the chromosomes in relation to those of the cells : maize chromosomes are small, hence their orientation in anaphase is less confined in space than that of the relatively large wheat chromosomes. In wheat it is invariably quite easy to identify

any fragment as originating from either a long or a short duplication ; there are no intergrades between them. This suggests different modes of origin for the two types of duplication. The question must be asked whether the long duplication ever arises from bridge breakage.

TABLE 1

*Crosses and selfings in the duplication strain*

| Parents | Number of progenies | LD/LD | LD/N | LD/SD | SD/SD | SD/N | N/N | LD/O | SD/O | N/O | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) 42-chromosome types | | | | | | | | | | | |
| N/N × LD/LD | 2 | ... | 18 | ... | ... | 3 | 2 | ... | ... | ... | 23 |
| N/N × LD/N | 8 | ... | 35 | ... | ... | 4 | 50 | ... | ... | 1 | 90 |
| N/N × LD/SD | 1 | ... | 3 | ... | ... | 5 | ... | ... | ... | ... | 8 |
| LD/LD × SD/SD | 1 | ... | 1 | 3 | 1 | ... | 1 | ... | ... | ... | 6 |
| LD/LD . . | 11 | 58 | 18 | 17 | 1 | 6 | 2 | 1 | 1 | ... | 104 |
| LD/N . . | 19 | 21 | 68 | 1 | ... | 11 | 55 | ... | ... | 1 | 157 |
| LD/SD . . | 4 | 4 | 6 | 14 | 7 | 7 | 1 | ... | ... | ... | 39 |
| SD/SD . . | 4 | ... | ... | ... | 20 | 10 | 1 | ... | 1 | 1 | 33 |
| SD/N . . | 10 | ... | 1 | 2 | 4 | 42 | 35 | ... | ... | ... | 84 |
| N/N . . | 5 | ... | ... | ... | ... | ... | 35 | ... | ... | ... | 35 |
| (*b*) Monosomics | | | | | | | | | | | |
| LD/O × N/N . | 2 | ... | 9 | ... | ... | 1 | 9 | ... | ... | 18 | 37 |
| N/N × LD/O | 3 | ... | 13 | ... | ... | 2 | 2 | ... | ... | ... | 17 |
| N/N × SD/O | 2 | ... | ... | ... | ... | 9 | 3 | ... | ... | ... | 12 |
| N/O × N/N . | 2 | ... | ... | ... | ... | ... | 15 | ... | ... | 21 | 36 |
| N/N × N/O . | 2 | ... | ... | ... | ... | ... | 13 | ... | ... | ... | 13 |
| LD/O . . | 10 | 4 | 11 | ... | ... | ... | 1 | 63 | 7 | 18 | 104 |
| SD/O . . | 4 | ... | ... | ... | 4 | 5 | 2 | ... | 25 | 2 | 38 |
| N/O . . | 8 | ... | ... | ... | ... | ... | 30 | ... | ... | 101 | 131 |

*Note.*—N/N is either White Fife or Tuscan.

Information on this point can be obtained from the progeny of crosses and selfings of plants with the short duplication ; an LD chromosome must be the result of bridge breakage. Among 167 plants raised from selfed SD plants and from SD crossed with N/N, three plants were found which carried an LD chromosome. This proves that a long duplication may arise from bridge breakage.

Whenever an SD chromosome occurs as the result of an unevenly broken bridge, a chromosome with a corresponding short deficiency (SD*ef*) should arise with equal frequency. The deficient chromosome would, however, be indistinguishable from a normal one.

### (*b*) *Quantitative*

(1) *The proportion of functional crossover gametes.*—We have seen that, with rare exceptions, the chromosomes resulting from a chiasma in an inverted duplication are either normal (which possibly includes short-

deficiency chromosomes) or have a short duplication. The frequency of crossing-over is known from observations in meiosis (*cf.* Frankel, *loc. cit.*) ; it is now possible to relate this to the frequencies of functional non-crossover and crossover gametes. While meiotic observations were confined to male cells, zygotic ratios provide information on both sexes.

Evidence on the proportions of functional pollen grains carrying non-crossover and crossover chromosomes is available from LD/LD ♂ crossed with N/N ♀ and from LD/O selfed and crossed with N/N ♀ (*cf.* table 1).

(*a*) N/N ♀×LD/LD ♂. The proportions of functional non-crossover and crossover gametes were as follows :—

18 LD : 3 SD : 2 N

or 0·78 non-crossover : 0·22 crossover, against 0·64 : 0·36 observed in meiosis.

(*b*) LD/O selfed. Pollen grains lacking the ABC chromosome are inviable ; hence 41-chromosome plants in the progeny of selfed LD/O are derived from ovules without the ABC chromosome, fertilised by pollen grains with LD (non-crossover), SD or N (crossover) chromosomes. The observed proportions were :—

63 LD : 7 SD : 18 N

and hence the ratio of functional non-crossover : crossover pollen grains was 0·72 : 0·28, against 0·6 : 0·4 observed in meiosis.

(*c*) N/N ♀×LD/O ♂. This cross produces only 42-chromosome plants. The proportions of functional gametes were as follows :—

13 LD : 2 SD : 2 N

a ratio of 0·76 : 0·24, against 0·6 : 0·4 in meiosis.

If (*b*) and (*c*) are combined, the ratio of non-crossover : crossover gametes is 76 : 29 or 0·72 : 0·28, against 0·6 : 0·4 in meiosis. The difference is statistically significant ($P < 0{\cdot}01$).

By combining all data for LD ♂ gametes in (*a*) to (*c*), the frequency of male functional crossover gametes obtained is 0·27. It is now possible to use the progeny of selfed LD/LD for obtaining information on the corresponding frequency in the female gametes.

The observed zygotic frequencies (table 1) are compared with those calculated on the basis of a ratio of 0·27 functional crossover gametes in the male, and of an output of female gametes according to the following assumptions (table 2) :—

(i) The frequency in the female is the same as in the male, *viz.* 0·27.

(ii) There is no elimination of crossover gametes in the female, hence the ratio of functional gametes is identical with the gametic output in meiosis, *viz.* 0·36 (observed in pollen mother cells).

(iii) The frequency of functional female crossover gametes is 0·5 as in LD/O ♀.

The first assumption provides the best fit; yet the possibility that female gametes with broken chromosomes function at a higher rate than male gametes (assumption 2) is not excluded. This indeed is more than likely to be the case since selfed LD/LD plants are fully fertile, which could not occur if female gametes were eliminated at the rate of 25 per cent. required by assumption (1).

TABLE 2

*Zygotic types in the 102 offspring of selfed* LD/LD, *with frequencies according to assumptions 1-3, derived from male and female gametes formed with and without crossing-over in meiosis.*

| Female gametes \ Male gametes | Crossovers (N, SD) | Non-crossovers (LD) |
|---|---|---|
| Crossovers (N, SD) . . . . | N/N, SD/N, SD/SD<br>(1) 7·2<br>(2) 9·8<br>(3) *12·8* | LD/N, LD/SD<br>(1) 19·9<br>(2) 26·5<br>(3) *38·3* |
| Non-crossovers (LD) . . . | LD/N, LD/SD<br>(1) 19·9<br>(2) 17·6<br>(3) *12·7* | LD/LD<br>(1) 54·9<br>(2) 48·0<br>(3) *38·3* |

| | N/N, SD/N, SD/SD | LD/N, LD/SD | LD/LD | P |
|---|---|---|---|---|
| Observed . . | 9 | 35 | 58 | ... |
| Assumption 1 . . | 7·2 | 39·8 | 54·9 | >0·5 |
| Assumption 2 . . | 9·8 | 44·1 | 48·0 | <0·2 |
| Assumption 3 . . | *12·8* | *51·0* | *38·3* | <0·001 |

Evidence which in general confirms these results comes from selfed LD/N and SD/SD; but here the presence of N and SD as both non-crossover and crossover chromosomes renders adjustments necessary which reduce the significance of observations.

It is concluded that the frequency of male functional gametes with a chromosome broken in meiosis is 0·27, or about 70-75 per cent. of the output of crossover chromatids in meiosis, and that it is the same or slightly higher in female gametes. Approximately one-quarter of gametes with broken chromosomes are non-functional.

(2) *The point of bridge breakage.*—It has been shown above that the main distinguishable derivatives of broken chromatid bridges are SD and N type chromosomes. The distinction is based on the presence or absence of internal duplication pairing and hence is of a preliminary nature; for N type chromosomes may be expected to include any of the following: (*a*) true N chromosomes derived from a bridge with median breakage point; (*b*) chromosomes with deficiencies

(SD*ef*), corresponding to the duplication SD and resulting from the same breakage; and, possibly, (*c*) duplications too small to form an internal chiasma, and (*d*) the corresponding deficiencies. These chromosome types cannot be distinguished; but it is possible to obtain a general idea of the proportions of median or near-median breaks yielding types (*a*), (*b*) or (*c*), and sub-median breaks yielding SD and SD*ef*.

For this purpose the observed zygotic ratios are interpreted in the light of two alternative assumptions: the first that SD*ef* gametes are functional, the second that they are non-functional. In the first case there should be a frequency of SD*ef* corresponding to that of SD, the balance of N type chromosomes being the result of median breakage; in the second case all N type chromosomes derive from median breakage. In either case an unevenly broken bridge yields one SD and one SD*ef* (= N type) chromosome; but an evenly broken bridge yields two N chromosomes.

(i) N/N ♀×LD/LD ♂—3 SD/N : 2 N/N, or 3 SD : 2 N.
SD*ef* functional—2 N are SD*ef*; O even : 2 uneven breaks.
SD*ef* non-functional—1 even : 3 uneven breaks.

(ii) LD/LD selfed—2 N/N : 6 SD/N : 1 SD/SD.
Ratio of SD : N, in both sexes—8 SD : 10 N.
SD*ef* functional—8 N are SD*ef*; 1 even : 8 uneven breaks.
SD*ef* non-functional—5 even : 8 uneven breaks.

(iii) LD/LD selfed—17 LD/SD : 18 LD/N, or 17 SD : 18 N.
SD*ef* functional—17 N are SD*ef*; 1 even : 17 uneven breaks.
SD*ef* non-functional—9 even : 17 uneven breaks.

(iv) N/N ♀× LD/N ♂—35 LD/N : 4 SD/N : 50 N/N.
When allowance is made for non-breakage N chromosomes, the following proportions are obtained:—

SD*ef* functional—1 even : 4 uneven breaks.
SD*ef* non-functional—2 even : 4 uneven breaks.

Summarised, the ratios of uneven and even breaks are as follows:—

SD*ef* functional—0 : 2, 1 : 8, 1 : 17, 1 : 4.
SD*ef* non-functional—1 : 3, 1 : 1·6, 1 : 1·9, 1 : 2.

The conclusion is reached that the majority of broken chromosomes are the result of uneven bridge breakage, whether short deficiencies are viable or not.

## 3. MONOSOMICS

In the progenies of plants carrying an inverted duplication monosomics are not uncommon, no doubt owing to the failure of pairing of the BC arm and to the resulting high frequency of non-conjunction. Six 41-chromosome plants were found among 534 plants derived from selfings and crosses of 42-chromosome plants with LD or SD duplications. The meiotic behaviour of LD/O and SD/O has

been described in the previous paper. The special interest regarding their zygotic output lies in the differential rates of functional gametes, both between crosses and selfings, and between male and female gametes.

### (*a*) *Chromosome numbers*

The progenies from crosses and from selfings of the monosomics N/O, LD/O and SD/O are summarised in tables 1 and 4. Table 3

TABLE 3

*Monosomics : crosses * and selfings of the same plants*

| Parents | 42-chromosomes | 41-chromosomes |
|---|---|---|
| N/N×N/O (1/12) | 7 | 0 |
| reciprocal | 7 | 11 |
| N/O (1/12) selfed | 3 | 9 |
| N/N×N/O (1/17) | 6 | 0 |
| reciprocal | 8 | 10 |
| N/O (1/17) selfed | 5 | 16 |
| N/N×LD/O (25/8) | 10 | 0 |
| reciprocal | 12 | 7 |
| LD/O (25/8) selfed | 6 | 15 |
| N/N×LD/O (25/10) | 7 | 0 |
| reciprocal | 7 | 11 |
| LD/O (25/10) selfed | 4 | 8 |
| N/N×LD/O (126/4) | 12 | 0 |
| LD/O (126/4) selfed | 0 | 20 |
| N/N×SD/O (125/4) | 8 | 0 |
| SD/O (125/4) selfed | 7 | 8 |
| N/N×SD/O (126/1) | 4 | 0 |
| SD/O (126/1) selfed | 1 | 9 |

* In the crosses of N/O and LD/O, N/N is White Fife ; in those of SD/O, N/N is Tuscan.

TABLE 4

*Monosomics : summary of crosses and selfings*

| Crosses | | | Selfings | | |
|---|---|---|---|---|---|
| | 42-chromosomes | 41-chromosomes | | 42-chromosomes | 41-chromosomes |
| N/N×N/O | 13 | 0 | ... | ... | ... |
| reciprocal | 15 | 21 | N/O | 17 | 69 |
| N/N×LD/O | 17 | 0 | ... | ... | ... |
| reciprocal | 19 | 18 | LD/O | 16 | 88 |
| N/N×SD/O | 12 | 0 | SD/O | 11 | 27 |
| 42×41 | 42 | 0 | ... | ... | ... |
| reciprocal | 34 | 39 | X/O | 44 | 184 |
| | *0·47* | *0·53* | | *0·19* | *0·81* |

gives details for those plants for which both crosses and selfings are available.

The transmission of the monosome differs in male and female gametes, as is evident from reciprocal crosses; and in crosses and selfings, as shown by the cross 41 ♀×42 ♂ *versus* selfed monosomics (table 4).*

Nullisomics for the ABC chromosome have failed to occur in the progenies of the selfed monosomics totalling 273 plants. This does not rule out altogether the possibility that nullisomics may be viable, since some occur in progenies of monosomics in wheat with a frequency as low as 0·9 per cent. (Sears, 1944). Yet, if they occur, their frequency is extremely low; and this, together with the lack of monosomics in crosses of 42-chromosome ♀×monosomic ♂, indicates that pollen deficient for the D chromosome does not function. There is no direct evidence for this interpretation, since the mature pollen of monosomic plants was found to be normally developed; yet the alternative assumption that nullisomics are eliminated through zygotic lethality requires that 20-chromosome pollen should fail to function with normal 21-chromosome ovules after crossing, but be fertile with its own 21-chromosome ovules after selfing. That this is improbable is shown below.

The evidence so far discussed is derived from progenies of selfed monosomics and of crosses of 42-chromosome ♀×monosomic ♂ (table 4). Here the analogy with other monosomes with inviable nullisomics is complete; pollen grains without the ABC chromosome fail to function; ovules are produced at the rate of four with 20-chromosomes to one with 21-chromosomes. It was found, however, that this ratio does not apply when a monosomic ♀ was crossed with a normal ♂. Here the ratio closely approximated one 20-chromosome ♀:

* The inheritance of a monosome depends on the rate of its inclusion in microspores and megaspores, on the viability of deficient gametes, on competition between normal and deficient pollen grains, and on the viability of the deficient zygotes. In hexaploid oats, three monosomes are transmitted equally through both sexes at rates between 6 and 14 per cent. Frequencies of inclusion in tetrad cells agree with zygotic frequencies. Nullisomics are viable, though some are semi-lethal (Nishiyama, 1931; Philp, 1935, 1938). In hexaploid wheat, Sears (1944) has obtained all 21 monosomics and 17 nullisomics. Female transmission, uniform for all monosomes, is about 25 per cent., the discrepancy from the possible 50 per cent. being due to loss of the univalent in meiosis; on the male side transmission varies from 1 to 15 per cent., the low value being attributed to competition with normal pollen. Watkins (1925) found a primary ratio in microspores of one-quarter to one-third lacking the univalent. He concludes that since the chance of a univalent being included in the innermost megaspore is less than random, the frequency of inclusion is likely to be lower on the female than on the male side. These primary ratios are modified either by partial pollen sterility or by slower pollen-tube growth of the defective grains. The case of the B series speltoids and fatuoids in wheat and oats, both monosomics, is more extreme. Here defective pollen as a rule fails to function; there are no or few nullisomics and an excess of monosomics on selfing (Nishiyama, 1931). In *Nicotiana Tabacum* all the 24 monosomics have been obtained, but no nullisomics. The primary ratio for microspores is about 80 per cent. defective to 20 per cent. normal for all monosomics, and the same is believed to occur on the female side; but differences in pollen and ovule development between the monosomic types result in a wide range of transmission rates in both sexes (Clausen and Cameron, 1944). Greenleaf (1941) found in a cytological study of embryo-sac development in *Nicotiana* monosomics that $n-1$ embryo-sacs had a slower rate of development than $n$, and also a higher frequency of abortion before reaching the eight-nucleate stage.

In diploid monosomics, defective pollen grains as a rule fail to function whereas eggs often are fertile. This is the case in *Nicandra physaloides* where pollen grains lacking the isochromosome die but deficient ovules are functional (Darlington and Janaki-Ammal, 1945).

one 21-chromosome ♀ (table 4). Two explanations are possible: (i) the true ratio of functional ovules is as in the cross, *viz.* 1 : 1. Then the ratio of 4 monosomic : 1 normal in the progeny of selfed monosomics requires that 20-chromosome pollen, non-functional in crossing, should be functional in selfing, with a frequency of three 20-chromosome : one 21-chromosome pollen grains (table 5).

TABLE 5

*Zygotic ratio from selfed monosomics, explained on assumption that defective pollen grains are functional on selfing but non-functional on crossing*

| Female gametes \ Male gametes | 3<br>20-chromosomes | 1<br>21-chromosomes |
|---|---|---|
| 1<br>20-chromosomes | [3]<br>40-chromosomes | 1<br>41-chromosomes |
| 1<br>21-chromosomes | 3<br>41-chromosomes | 1<br>42-chromosomes |

This assumption requires that three-eighths of the zygotes resulting from a selfed monosomic should be lethal. However, grain development of selfed monosomics is normal without any signs of partial sterility; and plant establishment in the progeny was 91 per cent. of the seed sown.

(ii) The true ratio of functional ovules in the monosomic is four 20-chromosome : one 21-chromosome. Then three-quarters of the 20-chromosome ovules, though functional with their own pollen, fail to function when crossed with 21-chromosome normal pollen. Here, therefore, there should be 60 per cent. lethal zygotes. Such lethality is hard to prove with grains resulting from crossing where seed setting is always uncertain; and plant establishment was normal, at the rate of 90 per cent. of the seed sown.

Both explanations assume differential compatibilities: the first, that 20-chromosome pollen is compatible with 21-chromosome ovules carried by the monosomic, but incompatible with those carried by a normal plant; the second, that the 20-chromosome ovules of the monosomics are more compatible with their own 21-chromosome pollen than with that of normal 42-chromosome plants. The evidence of ovule fertility disproves the first assumption; but the second is by no means established. It should be tested by using a N/N plant extracted from the duplication stock in place of White Fife. This is now in progress.

### (*b*) *Crossover frequencies*

It has been previously shown that the monosomic with the long duplication (LD/O) has an output of 0·24-0·28 of functional pollen grains with a crossover ABC chromosome, which is similar to the

output of the homozygote LD/LD (0·22). On the female side, there is evidence from crosses with White Fife ♂ (table 1). Monosomics derive their one ABC chromosome from the N/N male parent and are normal; 42-chromosome plants are LD/N, SD/N and N/N, in the proportion shown in the first line :—

| | Non-crossover | Crossover | | Ratio non-crossover : crossover |
|---|---|---|---|---|
| | LD | SD | N | |
| LD/O ♀ . | 9 | 1 | 9 | 0·9 : 1 |
| LD/O ♂ . | 13 | 2 | 2 | 3·3 : 1 |

The result of the reciprocal cross—which consists only of 42-chromosome plants—is shown in the second line. The figures are small, but the difference is significant ($P > 0{\cdot}01$). On the male side, the proportion of functional non-crossover and crossover gametes is about 3 : 1, on the female side about 1 : 1. It has been shown before that in the homozygote LD/LD the proportion of crossover ovules is between 0·27 and 0·36, but not 0·5 as in the monosomic LD/O. If the primary ratio in megaspore cells is the same in the monosomic as in the homozygote, then the deficiency of functional non-crossover ovules is due to differential elimination; but LD/O heads, which on this assumption should show partial sterility, are fully fertile.

This leaves the assumption that the primary ratio in LD/O megaspore cells is atypical. Here again there are two possibilities. (i) Internal duplication pairing is more frequent in the megaspores than in the microspores of the monosomic and in the megaspores of the homozygote. In view of the constancy of crossover values observed throughout this study, this seems improbable. (ii) A crossover chromatid has a greater chance than an LD chromatid of reaching the innermost megaspore cell.

Owing to the difficulty of obtaining the large numbers required for establishing quantitative differences, no observations on megaspore development have been made; but whatever the cause, the data indicate preferential segregation favouring the inclusion of crossover chromatids in the egg.

From the absence of inversion crossovers in *Drosophila*, Sturtevant and Beadle (1936) concluded that a first division bridge remains intact so that crossover chromatids are excluded from the terminal nucleus which forms the egg. Darlington and La Cour (1941) obtained cytological confirmation in *Lilium* and *Tulipa*, and, recently, Carson (1946) in *Sciara*. The former authors point out, however, that the non-inclusion of inversion crossovers does not apply where a chiasma proximal to the inversion causes the formation of a second division bridge. That in the present study there is no differential

distribution of crossover chromatids resulting from second division bridges is confirmed by the similarity of crossover ratios in the pollen grains and ovules of the homozygote LD/LD. The monosomic, however, shows preferential inclusion of crossover chromosomes in the ovule, the opposite in fact of the Sturtevant and Beadle effect. Here, as in the case of preferential segregation of knobbed chromosomes in the megasporogenesis of maize (Rhoades, 1942 ; Longley, 1945), no explanation can be given.

## 4. TELOCENTRICS

Telocentrics involving the duplication chromosome have been found independently on two occasions. In both instances the duplication arm was missing. Both were found as heterozygotes in the progeny of normal plants ; in the first the normal partner chromosome carried the long duplication, in the second it carried the short duplication (fig. 1). In the latter case the ABC chromosome

(*a*) (*b*) (*c*)

FIG. 1.—Telocentric, with BC arm missing, paired with SD : (*a*) metaphase I. (*b*) anaphase I. (*c*) complete metaphase cell with telocentric bivalent as above and another homozygous telocentric bivalent. × 1280.

was not the only telocentric. A telocentric bivalent was found side by side with the AB telocentric (fig. 1*c*). This pair usually forms a chiasma. The majority of the progeny carried this second telocentric as a bivalent, the remainder as a univalent. At the time of its discovery, preparations of some of the antecedents had been discarded ; yet the origin of the AB telocentric could be traced to the two preceding generations, since the third, and all sibs of the original plant, 29/7, and of its parent, were normal.

The parents and descendants of the two AB telocentrics were as follows :—

(*a*) 126/2 (1941-42) : LD/O

| Plot 31 (1943-44) : 1 N/N, 2 LD/O, 1 LD/TC * |

| Plot 22 (1944-45) : 1 N/N, 1 LD/SD, 1 LD/N, 1 LD/TC |

(*b*) 6/12 (1944-45) : SD/N

| Plot 29 (1945-46) : 1 SD/SD, 3 SD/N, 1 SD/TC * |

| Plot 2 (1946-47) : 4 SD/N, 3 SD/TC, 1 N/N, 1 N/TC,* 1 SD/O |

The expectation in the progeny of a heterozygote for a telocentric is 0·25 normal : 0·5 heterozygous telocentric : 0·25 homozygous telocentric. In the two progenies of heterozygous telocentrics the proportions were as follows :—

3 : 1 : 0
5 : 4 : 0

The absence of homozygous telocentrics and the deficiency of heterozygotes, together with the inviability of pollen lacking the ABC chromosome, suggest that it is the BC arm itself which is required for the pollen to be functional.

An AB telocentric may arise through misdivision of the centromere (Upcott, 1937*b* ; Darlington, 1940) in a univalent or non-conjoined bivalent ; and possibly through breakage of a dicentric chromatid at one of its centromeres. The former is frequent in LD/O and SD/O plants. Bridge breakage at or near a centromere has been observed (fig. 3) ; it can be inferred from the occurrence of the long duplication in the progeny of short-duplication plants.

Misdivision, telocentrics and isochromosomes are of frequent occurrence in wheat monosomics as well as in types with incomplete pairing (Love, 1940, 1943 ; Chin and Chwang, 1942 ; Sears, 1944, 1946 ; Smith, 1947). Sears found telocentrics for one or both arms in fifteen of the seventeen identified monosomics in common wheat. Thus in general polyploidy provides a wide safety margin, and the high degree of intolerance in relation to the BC arm deficiency is the exception rather than the rule ; and even in this instance where a deficiency is a pollen lethal, an excess has no apparent effect. However, as expected, in the diploid *Triticum monococcum* deficiencies and duplications arising from chromosome fragmentation, have serious physiological effects on size and other body characters, on fertility, and on gametic output (Smith, *loc. cit.*).

## 5. THE CHIASMA CYCLE AND THE ORIGIN OF THE INVERTED DUPLICATIONS

### (*a*) *The chiasma cycle*

It has already been shown that short-duplication and normal plants occur in the progeny of long-duplication plants, and, as a rare event, long-duplication plants in that of short-duplication ones.

* LD/TC = bivalent consisting of an ABC chromosome with the long inverted duplication and one lacking the duplication arm ; similarly, SD/TC and N/TC.

It is now possible to describe the system which leads from a duplication in one generation to a duplication—but not necessarily the same—in the next, a repetitive process of structural change which simulates the orderly process of hereditary transmission.

Fig. 2 illustrates the chiasma cycle which is found in the BC arm of *Triticum*. A chromatid bridge breaks either at the centre, (*a*), near the centre, (*b*), or at or near one of the centromeres, (*c*). Break (*a*) yields two normal chromatids, break (*b*) a short duplication and a corresponding deficiency, break (*c*) a long duplication and a telocentric (or acentric).

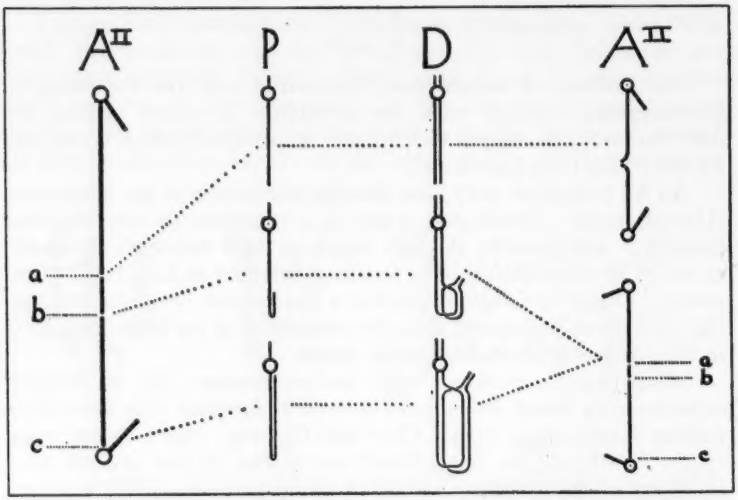


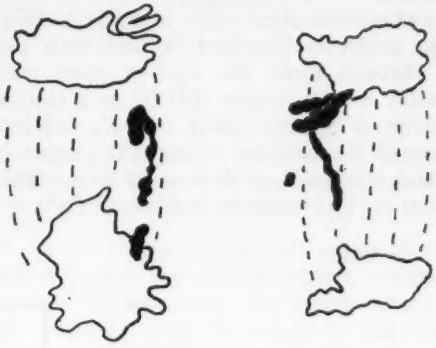

FIG. 2.—The chiasma cycle.

The new duplications—short or long—in turn are liable to be involved in inversion chiasmata, the majority of which, as has been shown in the previous paper, result in the elimination of the duplication as a fragment. When this occurs a bridge is formed thus closing the cycle, for this bridge breaks in (*a*), (*b*) or (*c*).

The frequencies of these events cannot be directly ascertained since the point of bridge rupture cannot be determined with precision by observation in anaphase. The functional gametes which carry chromosomes derived from bridge rupture can, however, be found in the next generation. It has already been shown that 27 per cent. of pollen grains carry a chromosome broken in the previous meiosis and that the proportion is the same or slightly higher in the ovules. The majority of breaks were found to occur near the centre—within less than one-tenth of the distance between the centre and a centromere—and the remainder at the centre itself.

That bridges are broken at—or very near—a centromere has been

seen in first-division bridges and in univalent bridges (fig. 3) ; centromere breakage of second-division bridges would scarcely be observable. Weakness at the centromere is not uncommon. Breakage at the centromere in anaphase, giving a telocentric and an apparent acentric, was found in *Tradescantia*, and centromere breakage of bridge chromatids in *Tulipa* (Darlington and Upcott, 1941*b*). Centromere weakness in metaphase of meiosis was also revealed by both X-ray and mustard gas treatment (Darlington and Koller, 1947).



(*a*) (*b*)

FIG. 3.—Bridge chromatids in anaphase I broken at or near a centromere : (*a*) univalent bridge, × 1700 ; (*b*) first-division bridge, × 1470.

More precise evidence comes from plants carrying the long duplication which are found sporadically in progenies of short-duplication plants. This occurred three times among the 167 plants identified in progenies of SD plants and in crosses SD × normal. An SD/N plant, 32/10 (1943-44), had the following progeny :—

4 N/N, 5 SD/N, 1 SD/SD, 1 LD/SD, 1 LD/N

The third exceptional plant occurred in the progeny of 24/13 (1945-46), another SD/N plant :—

1 N/N, 3 SD/N, 1 LD/SD

The exact frequencies with which broken bridges give SD and LD gametes cannot be stated, since crossover and non-crossover SD are indistinguishable ; but if the frequencies of SD gametes in the progenies of LD/LD, LD/N and LD/O are used to estimate the frequencies of SD crossovers in the corresponding SD/SD, SD/N and SD/O progenies, their number is calculated as 17·1, against the observed frequency of 3 LD gametes. Hence of the bridges which are broken unevenly, about six-sevenths break near the centre of the bridge and one-seventh at one of its centromeres.

### (b) *The origin of the duplications*

Three sets of direct evidence are available. First, the distribution of the duplication when discovered; second, observations in $F_1$ and $F_2$ from the cross of the parent varieties, Tuscan and White Fife; and third, the occurrence of short duplications in the progeny of long-duplication plants, and of long duplications in that of short-duplication plants.

In 1941-42, when the first cytological examination was made, seed from plants grown in 1935-36 was used, all of which belonged to the progeny of a single plant raised in 1934-35. The identification of the 1941-42 progenies identified in turn their parent plants of 1935-36 and, through these, the 1934-35 plant from which they derived—probably a heterozygote (LD/N) or a monosomic (LD/O). Nothing is known of its sister plants; hence our information does not extend beyond the presence of one LD gamete in the 1933-34 generation, which was the $F_5$ of the original cross. The reconstructed family tree, from 1933-34 onwards, is shown in fig. 4.

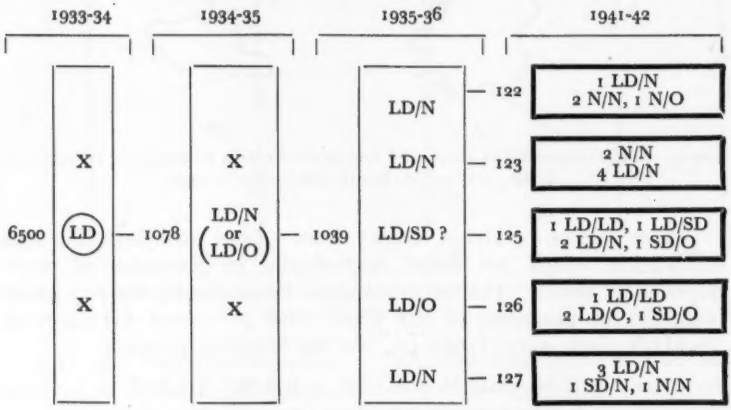



FIG. 4.—The derivation of the long duplication. Heavy frames observed, light frames inferred.

Seed of earlier generations was no longer available. Hence the original cross, Tuscan × White Fife, was repeated in 1943-44, and the $F_1$ and $F_2$ were examined. Chromatid bridges were found in both meiotic divisions of both generations. With the exception of one b' with which a very small fragment was found, visible fragments were absent. The frequencies are given in table 6.

The nature of these bridges cannot be defined with certainty. It is unlikely that they are due to crossing-over in small distal inverted segments, as inferred by Sax (1937) in *Tradescantia* and by Darlington (1937) in *Rhoeo*, since second-division bridges are too frequent to be accountable by inversion crossing-over. Moreover, the occurrence of

sporadic second-division bridges in N/N and other types suggests a common cause.

TABLE 6

*Frequencies of first and second division bridges in parents, $F_1$ and $F_2$*

| Plant | First division | | | Second division | | |
|---|---|---|---|---|---|---|
| | Number of | | Bridge frequency | Number of | | Bridge frequency |
| | cells | bridges | | cells | bridges | |
| Tuscan | ... | ... | ... | 990 | 0 | 0·00 per cent. |
| White Fife | ... | ... | ... | 980 | 1 | 0·10 ,, |
| $F_1$ | 114 | 1 * | 0·88 per cent. | 1323 | 9 † | 0·81 ,, |
| $F_2$ | 133 | 1 | 0·75 ,, | 1031 | 5 | 0·49 ,, |

* Bridge with very small fragment ; all other bridges without fragments.

† One of the nine had two bridges, the remainder one.

In addition to the chromatid bridges resulting from chiasma formation in the defined types of duplications, bridges with which no visible fragments are associated are found infrequently though regularly in all cytological types examined in this study. Bridges of this type occur mainly in anaphase II. They can be identified with certainty in types without inverted duplications and, in duplication types, in those cells in which they coincide with bridges resulting from crossing-over in the inverted duplication. Of 129 normal plants derived from the duplication strain, 71 had no bridges in counts varying from 50 to 100 anaphase II cells ; 58 had bridges, with an average frequency of 3 per cent. and a maximum of 10 per cent. In all normal plants, bridge frequency in 5152 cells was 2 per cent. ; 98 cells had single bridges and two had two bridges.

Is it likely that some at least of these bridges are due to crossing-over in inverted duplications too small to produce a visible fragment? This is unlikely to apply to all bridges without fragments. The bridge frequency for the long duplication is 40 per cent., for the short duplication 32 per cent. ; since the latter often approaches the limit of visibility, it is improbable that frequencies for a slightly shorter duplication would fall off to less than one-tenth.

It is in fact not possible to ascertain whether any of the bridges without visible fragments are formed by the duplication bivalent. That they are not confined to this bivalent is established, since in long-duplication heterozygotes, anaphase II cells with two bridges and one fragment, and in a homozygote a cell with three bridges and two fragments have been observed. In a monosomic a cell with three bridges and one fragment was found.

The alternative assumption of spontaneous sister reunion is consistent with the observed facts ; it is also consistent with observations

in meiosis of a variety of organisms (*cf.* Darlington and La Cour, 1945, pp. 243*ff*). In itself neither assumption is of direct relevance; for if, as is suggested, the inverted duplications originate from unequal bridge rupture, the fact that bridges occur is relevant rather than the cause of their occurrence.

Between the rare bridges in $F_1$-$F_2$ and the LD gamete in $F_5$ there is a gap in our direct evidence. Yet the facts presented in the preceding section help to bridge it. First, short duplications arise regularly from broken bridges; nearly one-half of all functional gametes resulting from bridge rupture have a short duplication. Secondly, long duplications arise also from broken bridges, though only one-seventh as often as the short duplications. This suggests that the duplications arose from bridges—presumably of the type observed in $F_1$ and $F_2$—by means of a two-phase process: first, a sub-median break, originating a short duplication, resulted in raising the level of bridge frequency; and secondly, as a sequel, a centric break produced the long duplication.

### (c) *The healing of broken chromatid bridges*

In maize, McClintock (1938, 1939, 1941) found that chromatid bridges broken in meiosis fail to heal in endosperm and in gametophytic tissue, forming a breakage-fusion-bridge cycle which is ended only by fertilisation when as a rule broken chromosome ends heal. *Narcissus bulbocodium* is apparently a more extreme case: here healing does not take place at all, even after years of breakage and fusion in somatic tissue (Fernandes and Neves, 1941).

In *Triticum*, as in maize, chromosomes broken in a meiotic anaphase are found in functional gametes. Contrary to the observations in maize, however, broken chromosome ends fail to fuse. No bridges were found in the first pollen grain mitosis. If every pollen grain carrying a broken chromosome were to form a bridge in the following mitosis, the frequencies of bridges would be 0·2 in LD/N and 0·36 in LD/LD (*cf.* Frankel, 1949). The number of cells examined and the number of bridges expected on the assumption of a breakage-fusion-bridge cycle are as follows:—

| | Number of cells observed | Number of bridges expected |
|---|---|---|
| LD/N . . | 85 | 17·0 |
| LD/LD . . | 49 | 17·6 |
| Total . . | 134 | 34·6 |

The probability that the absence of bridges is due to chance is infinitely small.

Thus in wheat, unlike maize, there is no sister reunion of chromatids broken in meiosis; healing apparently is rapid and permanent. This

is not surprising, for, as Darlington and Upcott (1941*b*) have shown, " healing of sister ends is variable and is both internally and externally conditioned." The nature of these conditions which, as is now seen, are sufficiently specific to cause healing of broken ends in one, sister reunion in another homologous tissue of related forms, is yet to be determined.

## 6. THE REPRODUCTIVE CYCLE

The preceding sections have dealt with the origin, the gametic output and the inheritance of the inverted duplications. It is now possible to approach the questions of their distribution in successive generations of a population and of their possible role as an evolutionary mechanism.

The zygotic output of the duplication types (*cf.* table 1) allows to predict the fate of the duplications, provided assumptions are made regarding the survival and reproductive rates of the types involved. It is assumed (1) that the proportions found in this study are a true expression of the proportions occurring in the progenies

TABLE 7

*Percentages of cytological types in successive generations in the progeny of* LD/N *(see fig. 5)*

| Generation | LD/N | LD/LD | LD/SD | LD/O | SD/N | SD/SD | SD/O | N/N | N/O |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 100·0 | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | 43·31 | 13·38 | 0·67 | 0·0 | 7·0 | 0·0 | 0·0 | 35·03 | 0·67 |
| 3 | 21·26 | 13·33 | 2·88 | 0·13 | 7·42 | 0·58 | 0·13 | 53·55 | 0·81 |
| 4 | 12·03 | 10·69 | 3·52 | 0·21 | 6·66 | 1·36 | 0·24 | 64·47 | 0·81 |
| 5 | 7·78 | 8·03 | 2·13 | 0·23 | 5·92 | 1·92 | 0·32 | 72·79 | 0·81 |
| 6 | 5·18 | 5·75 | 2·27 | 0·22 | 4·97 | 1·94 | 0·36 | 78·45 | 0·79 |
| 7 | 3·68 | 4·15 | 1·91 | 0·19 | 4·23 | 1·91 | 0·37 | 82·77 | 0·76 |
| 8 | 2·69 | 3·02 | 1·49 | 0·15 | 3·59 | 1·78 | 0·35 | 86·22 | 0·72 |
| 9 | 1·98 | 2·20 | 1·13 | 0·12 | 3·02 | 1·43 | 0·32 | 89·17 | 0·67 |
| 10 | 1·47 | 1·62 | 0·46 | 0·09 | 2·46 | 1·28 | 0·28 | 91·70 | 0·66 |

TABLE 8

*Cytological types in the fifth generation from an* LD/N *plant*

| | LD/N | LD/LD | LD/SD | LD/O | SD/N | SD/SD | SD/O | N/N | N/O | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Observed | 1 | 1 | ... | ... | 4 | 1 | ... | 29 | ... | 36 |
| Expected | *2·80* | *2·89* | *0·77* | *0·08* | *2·13* | *0·69* | *0·12* | *26·20* | *0·29* | *35·97* |

$\chi^2 = 1{\cdot}08$ ; $P < 0{\cdot}30$

of the various types ; (2) that these remain constant from generation to generation ; (3) that all plants survive to seed production ; (4) that all types have equal rates of reproduction. Then, starting with the type LD/N, the proportions of the various types up to the tenth generation are shown in table 7, and the proportions of LD

and SD arms in fig. 5. It will be seen that the frequency of the long duplication declines first rapidly and later more gradually, and that the short duplication, after an initial rise, follows the trend of the former. In extending the graphs beyond the tenth generation it is found that the long duplication will soon be extinct, survived by the short duplication by only a generation or two.

In a population derived from an LD/N plant whose progeny was grown without selection for 4 generations, 36 plants, taken at random,

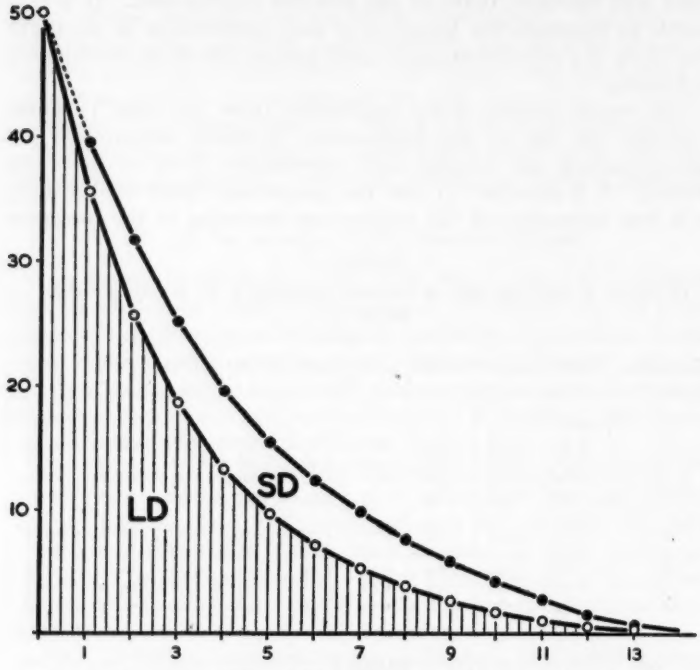



FIG. 5.—Frequencies of LD and SD gametes expected in successive generations in the progeny of LD/N.

were examined in the fourth. The proportions of cytological types are compared with the expected ratios in table 8. The small representation in all duplication classes, necessitating their being combined, reduces the significance of $\chi^2$; yet the fit of observed and expected ratios is striking. This confirms the validity of our assumptions. In particular it shows that the observed duplications have little if any physiological significance. It also proves that, left to themselves, they are removed rapidly and completely.

The persistence of an inverted duplication—the duration of the reproductive cycle—will depend on the rate of duplication crossing-over, internal as well as fraternal, and on the selective value of the

duplication. The case reported here is extreme in exhibiting very nearly the maximum rate of crossing-over combined with an absence of selective advantage. Crossover values in maize (McClintock, 1941) are similarly high. It remains to be seen whether they are substantially lower in other organisms. This in fact, should it be the case, would explain why inverted duplications have not been discovered more frequently. It is possible that duplications occur which are below the minimum size for chiasma formation in inverted segments, yet there is no evidence for this, and it should be noted that in *Triticum* even the short duplication has a high chiasma frequency.

If crossing-over in an inverted duplication occurs at all, ultimately, however low its frequency, it is bound to eliminate the duplication unless the latter has a strong positive selective value or unless it is transformed or transferred by a secondary change such as inversion or translocation. Hence the shorter its duration, the smaller the chance of its ultimate survival.

If inverted duplications possess an evolutionary significance, their closest analogues are perhaps the supernumerary chromosomes (*cf.* discussions by Darlington and Upcott, 1941*a*, and White, 1945, pp. 118*ff*). Both are self-destroying: supernumeraries by failure of pairing and by structural changes, inverted duplications by crossing-over. Both are capable of enriching the chromosome complement, the former with centromeres, the latter with "repeat" sectors—"apart from polyploidy . . . the only known process which may lead to an increase of the number of genes in the germ plasm of organisms" (Dobzhansky, 1941, p. 129). Both owe their existence to accidents though of a widely different nature; both have their life cycles, though of a different order. Compared with the subtle and orderly adaptability of polygenic systems or even with the storage and release of gene mutations in inversions, both constitute crude evolutionary mechanisms; but if and when they come into play, they would constitute major and irreversible steps affecting the foundations of the genetic structure.

There is yet another possibility of an evolutionary role for inverted duplications. Major changes in the chromosomes rarely fail to have a profound influence on other parts of the chromosome complement. It is not too much to expect that large duplications, though themselves of a transitory nature, may be the cause of secondary and more permanent changes. They may in fact be yet another destabilising evolutionary mechanism. Evidence of a major genetic change which coincided with the *Triticum* duplications will be presented in a third paper.

## 7. SUMMARY

1. This paper presents evidence on the transmission and evolutionary history of the self-propagating inverted duplications which were described previously.

2. The origin of the duplications is traced back to rare bridges without or with minute fragments in the $F_1$ and $F_2$ of the cross in which the duplications were found. An uneven bridge rupture, resulting in a short duplication, would raise the bridge frequency in the offspring sufficiently for the rarer event of a centromere break to occur and produce a long duplication.

3. Homozygotes for the long duplication (LD/LD) produce functional gametes with long (LD), short (SD) and without (N) duplications, The SD and N gametes arise from uneven and even bridge rupture ; but the N gametes may include short deficiency as well as normal gametes.

4. The frequency of functional gametes from LD/LD with a chromosome broken in the previous meiosis is about 27 per cent. in the male and the same or slightly more in the female. The majority of breaks are uneven but close to the centre of the bridge.

5. SD/SD, SD/N and SD/O plants normally yield SD and N gametes, but LD gametes are produced as rare exceptions. These are believed to arise from bridge rupture at or near a bridge centromere.

6. Crossing-over removes the duplications ; but, by causing bridge formation, it is instrumental in initiating new duplications, not necessarily of the original length. Thus LD can give SD and *vice versa*. There is a " chiasma cycle " from generation to generation.

7. Monosomics LD/O, SD/O and N/O produce 20-chromosome ovules, but 20-chromosome pollen grains are either non-functional or very rare ; hence no nullisomics have been found.

8. Monosomics have different proportions of functional gametes in selfings and crosses : 20-chromosome ovules are more compatible with their own 21-chromosome pollen than with that from a normal 21-chromosome plant.

9. In the LD/O monosomic, functional gametes carrying a chromosome from crossing-over in LD constitute about one-quarter on the male side, but one-half on the female side. A crossover chromatid thus has a greater chance of reaching the functional megaspore cell than has one carrying the duplication : this is the opposite of the Sturtevant and Beadle effect.

10. A telocentric produced by the loss of the duplication arm has been found on two occasions ; it is lethal as a homozygote.

11. Whether inverted duplications have evolutionary significance, *i.e.* whether they have a chance of incorporation in the chromosome complement, will depend in the first instance on the cessation of inversion crossing-over, through a coincident structural or genetical change. The chance of such an occurrence will be proportional to the length of the reproductive cycle.

12. If a heterozygote for the long duplication (LD/N) is propagated without selection, the duplications are calculated to have a reproductive cycle of 13 generations for LD and a further one for SD (fig. 5). Random sampling in the fifth generation was in accordance with expectation.

13. The evolutionary analogies between inverted duplications and supernumerary chromosomes are discussed. The latter may be a source of centromeres, the former of repeats.

14. Inverted duplications may be yet another destabilising evolutionary mechanism. Evidence of a genetic change associated with duplications is brought in a third paper.

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# NUCLEAR DIFFERENTIATION IN THE POLLEN GRAIN

By L. F. LA COUR

*John Innes Horticultural Institution, Hertford, Herts.*

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## 1. INTRODUCTION

THE satisfactory differentiation of the pollen grain is necessary for its effective life, and therefore directly bears on all breeding experiments with flowering plants. Moreover it is under a genetic control of a most complex kind, derived partly from the diploid parent and partly from the haploid cell itself, where it is the most sensitive indicator of the effects of segregation. For these various reasons a precise understanding of the physiology of its development is needed by the plant breeder.

A wealth of evidence has accumulated in support of the belief that nuclear differentiation in the pollen grain of Angiosperms is dependent on polarity of development. The most notable work in this direction has been done by Sax (1935), Sax and Husted (1936) and Geitler (1935). Notwithstanding the excellence of this work, some of the underlying principles have remained obscure.

In the present work, the initial observations were connected with an anomalous behaviour found in dwarf pollen grains of a single clone of *Tradescantia bracteata*. From information so gained, and by comparison with other material, it is believed that a plausible explanation for nuclear differentiation has been provided. In order that the problem should be seen in clear perspective, a review of the previous relevant literature is included.

## 2. SURVEY OF PREVIOUS WORK

### (*i*) *Formation of the pollen grain*

The formation of the pollen grains, and their arrangement in the tetrad, is effected by the two meiotic divisions. It is generally accepted that the two second divisions in most monocots occur after the first-division wall is formed and on axes perpendicular to the first. Where the two axes are in one plane, the four nuclei are arranged in the same plane, with the pollen grains meeting along one diameter of the mother cell.

This mode of formation, which we may call type I, is of the kind ascribed to the diploid species of *Tradescantia* examined by Sax and Edmonds (1933), Sax and Husted (1936) and Johnson and Peck

(1937). The last, however, describe in one-fifth of the cells (or one-half in *Rhoeo discolor*) an alternative kind of tetrad formation which, although not mentioned, was doubtless seen by Sax and his collaborators. It is derived from the two spindles at the second division being in axes at right angles to each other. It was concluded, that this less frequent type of division, which we may call type II, was localised in small sectors of the anthers.

In a more recent contribution, Vogl (1947) has surveyed the different kinds of tetrad formation seen in various monocots. She finds that there is very generally, some deviation from the two kinds of second-division behaviour just described. Cells occur, in which the two spindles are orientated at intermediate angles, to give intermediate kinds of tetrad formation between types I and II. She concludes that spindle orientation is influenced by cell shape, cell pressure, size of the spindle in relation to cell size, and sometimes by " internal cytoplasmic action."

In the tetrads of dicots, a tetrahedral arrangement of cells is commonly found. It arises from the two second divisions taking place in planes at right angles to each other as in type II of the monocots, but with partitioning of the cytoplasm simultaneously with completion of the two meiotic divisions. Such an arrangement occurs in some species of monocots, *e.g.* in *Gasteria* and *Aloë* (Johnson and Peck, 1937).

### (*ii*) Development of the free pollen grain

Normally, in most Angiosperms, the pollen grains are released from the tetrad before they undergo mitosis. Exceptions to this common behaviour, are found in most of the *Ericaceæ*, e.g. *Erica persoluta* (Geitler, 1935) ; all members of the *Juncaceæ*, e.g. *Luzula purpurea* (Malheiros, Castro and Câmara, 1947) ; *Elodea* (Wylie, 1904) and *Salpiglossis sinuata* (Wodehouse, 1935). In the *Orchidaceæ*, in some species, the pollen grains are found in tetrads while, in others, several hundred pollen grains remain in packets which are loosely united to form the pollinium (Barber, 1942). A similar situation to the last occurs in the *Mimosaceæ* and *Asclepiadaceæ*.

With few exceptions, a vacuole arises early in development, toward one end of the pollen grain, (*cf.* Wulff and Maheshwari, 1938). Vacuole formation is normally absent in *Luzula purpurea* (Malheiros, *et al.*, 1947) and in some species of *Haworthia* (Dr Pinto Lopes, personal communication). In the last species, a dense mass of cytoplasmic granules, may possibly as we shall see, have the same effect.

In *Tradescantia* the activity of the vacuole appears to be closely connected with the disappearance of the cytoplasmic granules. These range from about 0·5-1 $\mu$ in diameter and are thought to be of a protein nature. They occur at meiosis and, persist in the pollen grain, till formation of the vacuole (Sax and Edmonds, 1933).

The presence of the vacuole leads to a migration of the nucleus

and a confinement of most of the cytoplasm to one end of the cell. In some species, however, there may be a further movement of the cytoplasm and nucleus, prior to pollen-grain mitosis. This happens where the *dorsal* wall, originally the *inner* wall in the tetrad, flattens out, *e.g.* in *Tradescantia* (Sax and Edmonds, 1933).

In these species following the first movement, and at about the time of early prophase, there is a movement of cytoplasm and vacuole, so that two vacuoles are formed ; one at each end of the cell, with the bulk of the cytoplasm between them. The nucleus then lies central in the long axis of the cell, but toward the dorsal wall.

### (iii) *The pollen-grain mitosis*

The position of the mitotic spindle, and its angle to the wall, is under normal conditions constant usually for a whole species and sometimes for a whole genus (Geitler, 1935 ; Sax and Husted, 1936). The last-named believe, that with few exceptions, the spindle is at an angle of 45° to the axes of both meiotic divisions.

The spindle is, as a general rule, short. It is also asymmetrical being closer to the side at which the generative nucleus will be cut off. This leads to a difference in arrangement of the chromosomes at the two poles. From the work of Brumfield (1941) it is evident that the degree of asymmetry is associated with the position of the prophase nucleus, asymmetry being greater where the nucleus is flattened strongly on the side adjacent to the wall.

It is also evident that the degree of asymmetry can be variable, for Hagerup (1938) in *Orchis maculata*, found the metaphase spindles to be symmetrical but those of anaphase and telophase asymmetrical. On the other hand, Brumfield from observations in *Allium*, *Pancratium* and *Tradescantia* concluded that the degree of asymmetry decreased from metaphase to anaphase. My own observations in *Tradescantia*, as we shall see, show that at telophase the spindle is usually more asymmetrical than at anaphase. These observations also show that the shape of the spindle is influenced by vacuole formation, which we know, from the observations of Sax, is influenced by environment. There may thus be an explanation for the isolated claims that the spindle is symmetrical in some species (*cf.* Maheshwari, 1949).

The spindle is also asymmetrical where it extends through the whole depth of the cytoplasm, as in *Uvularia* (according to Geitler's illustrations). Thus, irrespective of spindle length, asymmetry is doubtless responsible for the resulting cells being unequal (*cf.* Wulff and Maheshwari, 1938).

From the work of Sax (1935) it is evident that the position of the generative nucleus, and indeed its differentiation, is governed by the position and angle of the mitotic spindle. Geitler (1935) has shown that the generative nucleus, in a large majority of species, is cut off

towards the wall which was inmost in the tetrad. Reported departures from this behaviour, are :—

| | | |
|---|---|---|
| (i) Radial wall | *Allium* | Geitler, 1935 |
| (ii) Outer wall | *Elodea* | Wylie, 1904 |
| | *Vaccinium* | Samuelsson, 1913 |
| | *Albizzia* | Maheshwari, 1931 |
| | *Acacia* | Newman, 1934 |
| (iii) Facultative | *Acanthus spinosus* | Drahowzal, 1936 |

In *Acanthus* it can apparently be cut off, at either the inner, or outer wall. Likewise, in *Periploca sepum* the position varies, but this is attributed to the shape of the pollen mother cells influencing the direction of the second-meiotic divisions (Sax and Husted, 1936).

As Wulff and Maheshwari (1938) have pointed out, the germ pores and furrows often serve as useful markers. The exact position of the generative nucleus in many species must always remain obscure, owing to lack of markers. Its discovery is simplified of course, where the pollen grains remain united, naturally or unnaturally, in a common exine.

### (iv) Differentiation

*The nuclei.*—The pollen mitosis normally leads to the formation of daughter nuclei with equal amounts of chromatin, but of unequal size. From the very beginning the generative nucleus is recognisable by its smaller size. Later the distinction is made doubly clear by a marked difference in staining capacity between the two nuclei. The vegetative nucleus, in expanding, gradually loses its affinity to react with basic dyes. Apparently Wulff (1933) was the first to show its weak reaction to Feulgen's reagent. The generative nucleus on the other hand reacts strongly. It is generally assumed therefore, that the vegetative nuclei lose their desoxyribose-nucleic acid.

*The cytoplasm.*—It is well established that the cytoplasm on the two sides diverges in regard to staining. The generative cell loses its affinity towards all stains. The vegetative cell gains in affinity. They thus diverge also from their own nuclei. The change in the vegetative cell was noted by Painter (1943) in *Rhoeo discolor*, who found that the cytoplasm of the mature pollen grain is rich in ribose-nucleic acid. He suggested that it accumulated in the pollen grains at the expense of the tapetal cells.

### (v) The effects of environment

Sax (1935, 1937, 1942) has shown how nuclear differentiation is influenced by the environment : in *Tradescantia*, high and low temperatures induced the same end result : disturbed polarity and, consequently, failure of differentiation. In a triploid, unbalance likewise led to failure of differentiation.

Similarly Koller (1943) in *Tradescantia*, has shown that irradiation before mitosis suppresses differentiation. He also found, that the staining capacity of micro-nuclei depended solely on their position at the end of telophase. They followed that of nuclei to which they were adjacent.

Associated with errors in differentiation, the most frequent event is the supernumerary division of the vegetative nucleus (*cf.* Upcott, 1939). Sax records Mrs Sax as having found in *Picea* a pollen grain with four equal-sized nuclei. There are, however, similar kinds of behaviour attributable to other causes. In *Sorghum*, Darlington and Thomas (1941) found pollen grains with extra divisions of the vegetative nuclei. But here extra division was influenced by the inclusion of heterochromatic chromosomes supernumerary to the basic set.

The generative nucleus may sometimes divide precociously, that is in the pollen grain instead of in the pollen tube (Upcott, 1939). Following temperature shock it may likewise divide in the pollen grain, but without equational division of the chromosomes (Sax, 1937).

## 3. MATERIALS AND METHODS

The *Tradescantia bracteata* material consisted of four clones ($20^1$, $21^2$, $21^3$ and $22^1$) the remains of a collection provided by Dr L. F. Randolph in 1929. All clones regularly form six bivalents at meiosis. The following four plants were also examined for comparison: *Tradescantia virginiana* ($4x$), *T. canaliculata* ($2x$) × T. Hamburg ($4x$), *Scilla sibirica* ($2x = 12$) and *Luzula purpurea* ($2x = 6$).

Apart from a few observations in acetic stains and sectioned material, the anthers were usually smeared and fixed in acetic alcohol (1 : 3). Fixation was limited to 30 minutes, but hardening was continued by leaving the smears in 95 per cent. alcohol overnight prior to staining.

Following hardening, slides were stained: (i) in methyl-green pyronin, (ii) in 1 per cent. toluidine blue, (iii) by Feulgen's reagent, with and without counter-staining in the acid dye Ponceau red RR, or (iv) in the acid dye alone. The acid-dye solution, was prepared in the way proposed by Hydén (1934) for the study of protein metabolism in nerve cells. The other methods have been described in detail by Darlington and La Cour (1947).

## 4. DWARF POLLEN GRAINS

A chance examination of anthers, from late buds (with bi-nucleate pollen grains) of the clone $20^1$ (which I shall refer to as the sensitive clone), revealed a number of delayed uninucleate grains regular in size, but approximately two-thirds the volume of normal pollen grains. Counts made from single anthers of different buds showed that they represented 15-25 per cent. of the total pollen grains. In each case the remainder being of regular size. Little difference in the proportion

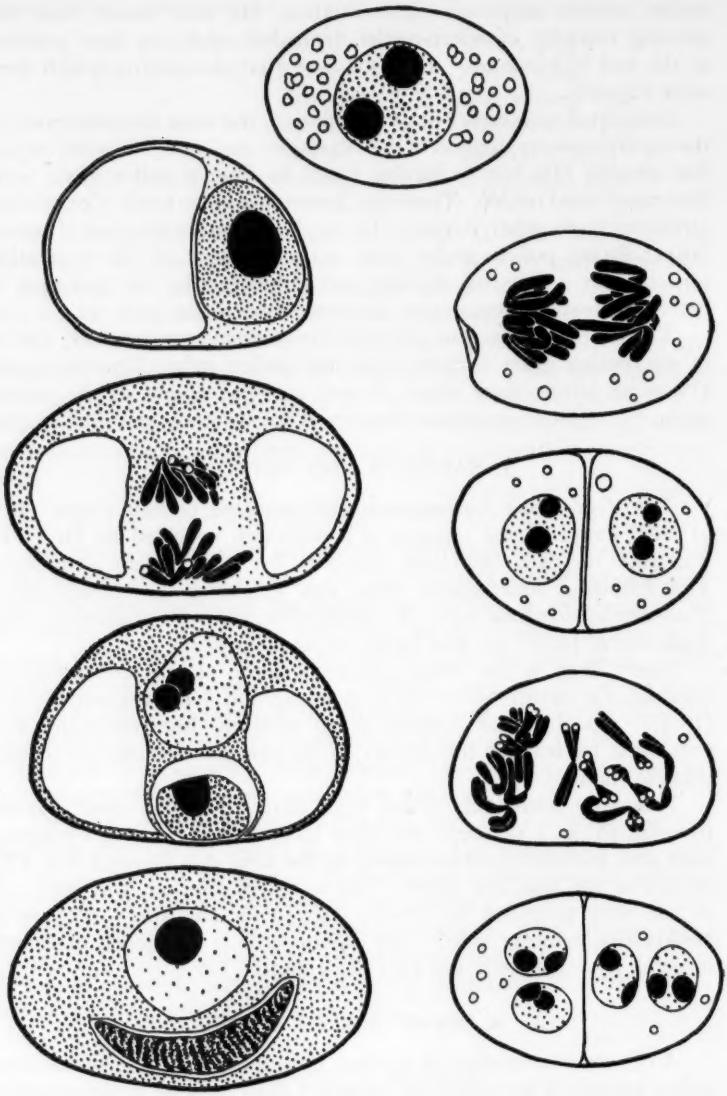


FIG. 1.—Stages in the development of normal and dwarf *pollen grains* in *Tradescantia bracteata*. Stippling indicates the concentration of protein and ribose-nucleic acid in the cytoplasm ; desoxyribose-nucleic acid in the nuclei. The extra mitosis in the dwarf grains occurs only after temperature shock (see later in the text). ×1500.

of dwarf grains was found between individual anthers in a bud. Great variation was found between buds. These first counts were made at the end of the 1945 season. Since then proportions have been found as high as 40 per cent. but never lower than the 15 per cent. There are no intermediate grains.

At mitosis, the dwarf grains always show the normal chromosome number, $2n = 6$. At anaphase, however, the division is almost invariably lengthwise in the cell instead of crosswise. Following division, the cytoplasm usually divides equally between the two telophase nuclei, which remain of equal size and staining density. With Feulgen staining, they show the nucleic acid deficiency of typical vegetative nuclei. Mitosis in the dwarf grains, begins at about the time it ceases in the normal-sized grains, and only about 40 per cent. of them manage to divide before the anthers dehisce.

The difference in size between the two kinds of pollen grain is first conspicuous at the end of mitosis in the normal pollen grain. Earlier than this, the dwarf grains are noticeable only by the absence of the vacuole. A comparative history of the two types (fig. 1) shows that vacuoles are never developed in the dwarf grains; but they retain the cytoplasmic granules, with some diminution for the greater part of their life. Two other characteristic features become apparent later: (*a*) The *dorsal* wall is less distinct, giving the cells a more symmetrical shape than normal pollen grains. (*b*) The cytoplasm appears thin and, possibly because of this, the walls sometimes partially collapse with mounting in "Euparal."

How is the origin of these dwarf grains to be explained? First, the extreme variability in the proportion of such grains excludes the view that they result from the recombination and segregation of two segregating genetic differences. Secondly, an alternative view that they might result from the consequences of inversion crossing-over is also excluded. The frequency of inversion crossing-over varied in the reverse direction to the proportions of dwarf grains determined at the same time, as shown in table 1, and was in any case far too low.

TABLE 1

*Frequency of inversion crossing-over in relation to the formation of dwarf pollen grains*

| | Total cells | B | B+*f* | *f* | 2*ff* | Joint | Inversion crossing-over | Dwarf pollen grains |
|---|---|---|---|---|---|---|---|---|
| 1945 A I | 213 | 5 | 5 | 3 | ... | 357 | 4·8 per cent. | 15 per cent. |
| 1945 A II | 144 | 1 | ... | 2 | 1 | | | |
| 1947 A I | 203 | 3 | 1 | ... | ... | 353 | 1·1 ,, | 40 ,, |
| 1947 A II | 150 | ... | ... | ... | ... | | | |

In 1947 an attempt was made to grow the clone 20[1] under controlled temperature conditions, in order to relate the effect of temperature

with the dwarf-grain reaction. These experiments failed presumably because of incorrect lighting. After 3-4 days treatment cell development stopped ; the pollen mother cells would not progress beyond meiosis.

Pollen-grain counts were then made from plants in the field and a record made of dates on which the material was fixed. Comparison with meteorological records, shows that sensitivity to the production of dwarf grains seems at times to be influenced by drought and possibly also by high temperature (table 2).

TABLE 2

*Frequency of dwarf grains in the sensitive clone 20¹ in relation to weather conditions*

| Period | | Temperature (over 12 days) | Rainfall (over 12 days) | Dwarf pollen grains |
|---|---|---|---|---|
| 1945 | October . . . . | ... | ... | 15 per cent. |
| 1947 | to July 12th . . . | 61-73 | 0·67 in. | 24 ,, |
| | to September 2nd . . | 72-81 | nil | 35 ,, |
| | to October 10th . . | 66-67 | ,, | 30 ,, |
| | to October 20th . . | 54-59 | 0·02 in. | 41 ,, |
| 1948 | to August 1st . . . | 69-94 | 0·08 in. | 41 ,, |
| | to August 17th . . | 64-71 | 2·0 in. | 34 ,, |
| | to August 24th . . | 64-72 | 2·3 in. | 38 ,, |

Included here, is one other observation on abnormal pollen-grain development which is worthy of note. Amongst a sample of pollen grains in *Scilla sibirica*, an anomalous case was found where four pollen grains had remained together in a tetrad. In three of them differentiation was complete. The fourth showed the consequences of derangement in polarity (fig. 2). It may also be mentioned that the pollen grains of this species contain numerous cytoplasmic granules, unlike those in *Tradescantia*, however, their activity appears in no way to be connected with that of the vacuole.

## 5. ORIENTATION OF THE SECOND MEIOTIC SPINDLES

The search for inversion bridges and fragments at meiosis in the clone 20¹, revealed a proportion of second anaphase cells, with a spindle orientation similar to that reported by Vogl. By visual observation alone, the cells within an anther could be classified into three groups, as we saw earlier, types I, II, and intermediate. Comparison of *T. virginiana*, a triploid hybrid, and the other clones, revealed a difference in the proportions of the three types of cells. In the triploid the frequency of intermediate cells was much higher. No doubt in triploids, spindle orientation is influenced also, by unequal chromosome distribution.

It was first considered whether cells of the intermediate type

might (together with the genotype of the sensitive clone) be held responsible for the origin of the dwarf grains. An attempt was then made, to detect by accurate means, any differences in spindle orientation that might occur between the clones.

By visual observation alone this is impossible. But if with a camera lucida, the focal points of the poles are projected, the angle between them can be determined, the unit readings on the fine adjustment providing the units of the co-ordinate system. It is essential to select only cells at late anaphase, and of equal development on both sides.

In this way, an analysis was made in clones $20^1$ and $21^2$. The smear used for analysis of the clone $20^1$, was from material grown at 63°-65° F. Unfortunately the analysis was made out of season, and no temperature-controlled experiments had been undertaken with clones without dwarf grains.

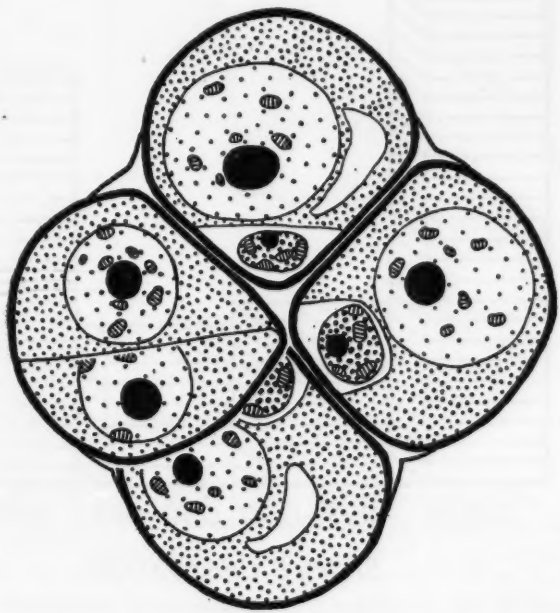


FIG. 2.—Anomalous coherence of pollen grains in *Scilla sibirica*, showing failure of differentiation in one grain through derangement in polarity of mitosis. Note, specificity in position of the generative nuclei. × 1300.

The proportion of dwarf grains varied, from 15 per cent. in a sample of 1945, to 41 per cent. in a sample of 1948. The proportion

of intermediate type spindles was 17·4 per cent. for clone 20[1] in the controlled temperature sample of 1947, and 25·4 per cent. for clone 21[2] at an uncontrolled temperature (fig. 3). The dwarf grains may therefore be derived from the intermediate type of cell with skew

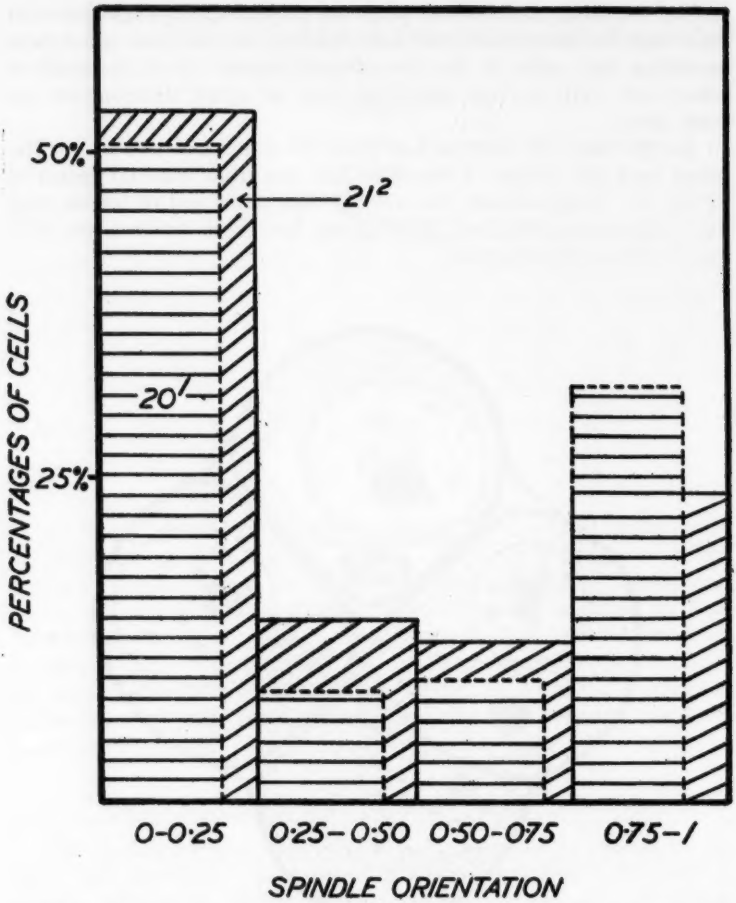



FIG. 3.—Graph showing an analysis of spindle orientation at the second meiotic anaphase in two clones of *Tradescantia bracteata*, obtained by measurement of the angle between spindles.

spindles, but controlled environmental observations, of the grains and the spindles, will have to be made together to test the hypothesis. Further information may be provided by breeding experiments, since some natural seedlings of the sensitive clone show the same sensitivity.

## 6. STAINING WITH PONCEAU RED AND METHYL-GREEN PYRONIN

At first Ponceau red was used purely as a counter-stain, in order to render the cytoplasm and vacuoles clearly visible after staining with Feulgen. The unstained vacuoles and refractive granules, are then shown in relief against the pale-red staining cytoplasm. Staining in the dwarf grains, however, was almost absent.

Slides were then stained showing together all stages of development from meiosis to late pollen grains. In this way, I hoped to obtain an early difference in stainability of the cytoplasm, and hence to trace

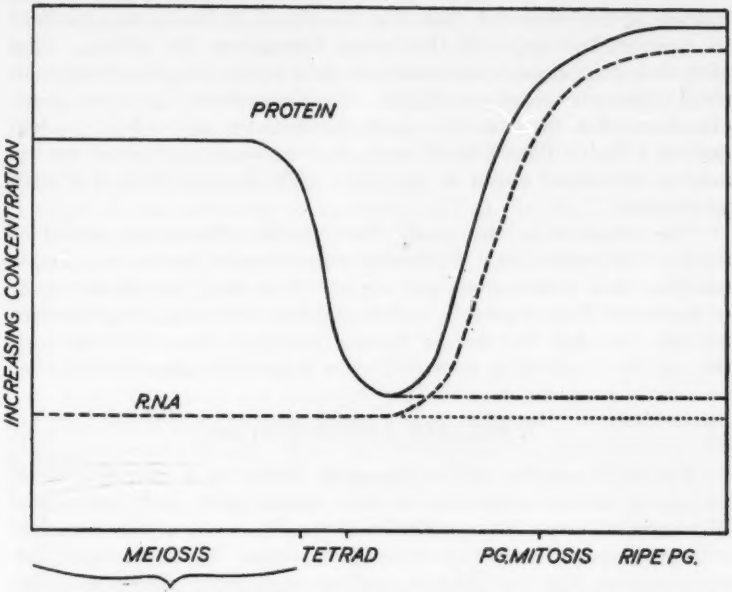



FIG. 4.—Graph showing the relationship between protein and nucleic acid concentration in the cytoplasm during the development of normal and dwarf pollen grains in *Tradescantia bracteata*.

the development of the dwarf grains back to its very origin. This I failed to do, but the results were interesting in connection with the staining of the cytoplasm at different stages of development. The stainability of the cytoplasm decreases at the time of the formation of the pollen grains, but increases correspondingly, with their growth and development. Similar results were obtained with Ponceau red alone as an acid stain. Similarly prepared slides were then stained in methyl-green pyronin in order to show the distribution of ribose-nucleic acid. The observations from both treatments are expressed in fig. 4.

How are these differences in reaction to be interpreted? It would be unsafe to assume, that the differences found with the acid dye, imply differences in basic substances which are correlated with

particular stages of development in the cell. It is safer to assume, since the reaction of any acid dye principally depends on adsorption, that the differences are connected with changes in density of the cytoplasm. These changes may possibly be connected with, in the first place, formation of the pollen grain, later with the growth and function of the free pollen grain. Staining with Ponceau red does, however, show one thing that appears to be certain. It shows that the dwarf grains are deficient in proteins.

Painter as we saw earlier, has suggested that ribose-nucleic acid is drawn by the pollen grain, from the tapetal cells. Sections of anthers in the clone $20^1$ show that the dwarf grains are not localised in position, but appear to be spread throughout the anther. That they do not accumulate ribose-nucleic acid in the same way as normal-sized grains may seem remarkable. But Caspersson (1940) has shown elsewhere that ribose-nucleic acid accumulates only where protein activity is high. Thus it would seem, that the low level of ribose-nucleic acid in the dwarf grains is associated with the low level of protein production.

The observation also made clear another important aspect of nuclear differentiation. Following either methyl-green pyronin or toluidine-blue treatment it was obvious that no ribose-nucleic acid, or extremely little, is present in the cytoplasm confining the generative nucleus. A clear but not so marked difference was also seen with the acid dye, indicating some difference in protein concentration.

## 7. NUCLEAR DIFFERENTIATION

The pollen-grain mitosis normally leads to a distribution of cytoplasm whose inequality is both quantitative and qualitative. The knowledge that the qualitative inequality is one of ribose-nucleic acid provides a simple explanation for nuclear differentiation. The cytoplasm on the two sides is working in opposite directions. On one side, protein and ribose-nucleic acid activity is high : the function being one of *growth*. On the other, ribose-nucleic acid is negligible with protein activity presumably low : the function being one of further *mitosis* for sperm production.

The unequal distribution is relatable only to the mitosis. The most plausible explanation is provided by the apparent asymmetry of the spindle and seemingly, its control of spindle length. In the normal pollen grains of the clone $20^1$, the spindle seldom extends to the whole depth of the cytoplasm. From mid-anaphase to early telophase, the spindle normally becomes increasingly asymmetrical and shorter in length. In the dwarf grains, however, the spindle is almost invariably long, with the poles equidistant from the walls. The spindle is therefore never asymmetrical (fig. 1, plate II).

In order to provide further evidence, material was examined, where failure of differentiation had been induced either by temperature

or X-rays. The effect of both kinds of treatment on development, is as we saw earlier, closely similar. They lead to an upset in cell processes such that many pollen grains occur with incomplete vacuole formation at the time of mitosis. Phases are then available, intermediate between accomplishment and failure of differentiation. I then found that when the angle of spindle orientation was deranged through incomplete vacuole formation, the degree of asymmetry was lessened. The pole adjacent to the wall moved inwards. The consequences were clear: when the disturbance in polarity was not too severe, the cytoplasm could still be unequally divided, but nevertheless nuclear differentiation was incomplete. It failed, as we shall see, because the quality of the cytoplasm was not right (fig. 5, plate II).

Observations in *Luzula purpurea* showed what the quality should be. In this species the four primary nuclei remain in a tetrad. They are retained in the original pollen mother cell wall and partitioning of the cytoplasm does not happen until differentiation is complete. The four mitoses are not always in step, and it is possible to compare changes in the character of the cytoplasm, at slightly different stages of development within a single cell. To avoid sectioning, iron aceto-carmine was used, but the accuracy of the observations was checked in methyl-green pyronin. No vacuoles are formed. The cytoplasm is extremely dense, and because of this, overstaining of the cytoplasm is difficult to avoid. Heavy staining, however, is necessary in order to trace the series of events.

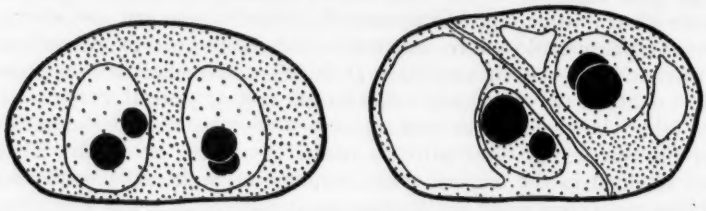
Four clear zones are markedly noticeable in the cytoplasm. It is in these zones that the spindles are formed, following the dissolution of the nuclear membranes. From pro-metaphase onwards ribose-nucleic acid infiltrates into them. At anaphase the outer poles are almost enclosed. With completion of the division only the inner poles have escaped the invasion. These yield the generative nuclei: they remain in clear cytoplasm (plate I). Thus it is evident that the cytoplasm received by the generative nucleus is comprised solely, or mostly, of nuclear sap derived from the primary nucleus. It is also clear that partial differentiation is related to the degree of contamination from the granular cytoplasm.

That the whole process is so clear in *Luzula*, is probably due to the absence of inner walls. In *Tradescantia* and presumably in most other plants, the clear cytoplasm is compressed tightly against the wall. It is only after telophase, when the spindle has dissolved, that what has happened becomes clear.

## 8. THE EFFECTS OF TEMPERATURE SHOCK

Potted plants of *T. bracteata*, clone 20[1], were kept at periods of 1-6 hours at 38° C. and then transferred to −5° C. for 1-2 hours. With each experiment, the first observations were made after an interval of 24 hours following the treatment.

Chromosome breakage was found once, and although severe, it was nevertheless confined to a single spike. The treated plants were examined periodically, however, in order to study the effects of the treatment on pollen-grain development. The observations revealed abnormalities similar to those found by Sax in his experiments. They included failure of differentiation in the normal large grains and anomalous division of vegetative nuclei.



(*a*) (*b*)

FIG. 5.—Pollen grains in *Tradescantia bracteata* after irradiation: (*a*) complete failure of differentiation, (*b*) partial differentiation. Stippling of the cytoplasm indicates differences in protein as shown by Ponceau red. × 1500.

In one sample, 20 days after treatment, some of the dwarf grains likewise, showed division of both daughter nuclei formed at the primary mitosis (fig. 1). In some cells the two divisions were not always strictly in step. Similarly, in other cells, the divisions were dissimilar in orientation. More frequently, however, both divisions were in the short axis of the cell. Partitioning of the cytoplasm usually followed anaphase separation, giving, where the divisions were parallel, pollen grains resembling tetrads (plate II).

## 9. CAUSAL SERIES OF VARIATIONS

From the foregoing it is evident that nuclear differentiation is dependent on a polarity of pollen-grain contents. It is brought about by movements and changes in the character of the cytoplasm. The principles involved and the variations in development which occur can be defined as follows :—

1. Orientation of spindles at the second anaphase of meiosis.
2. In *Tradescantia:* the disintegration of cytoplasmic granules. Failure to disintegrate leads to failure of vacuole formation.
3. Development of vacuoles (possibly connected with the germ pores since they are formed adjacent to them) leads to a spatial restriction of the cytoplasm.
4. Position of the primary nucleus related to :—
   - (*a*) as in *Haworthia*, the position of the vacuole or where it is absent, to the cytoplasmic granules ;
   - (*b*) a wall, causing it to be eccentric. It lies towards the thick wall in most species with single pollen grains, e.g. *Tradescantia ;* or in the centre of the pollen mother cell where the grains remain naturally united, e.g. *Luzula.*

5. Differentiation of cytoplasm into (i) dense granular type rich in imported ribose-nucleic acid and (ii) clear fluid type which must largely be composed of nuclear sap.

6. (*a*) Spindle always arises from clear cytoplasm. Thus the clear type is converted into a third kind of *fibrous* cytoplasm.

(*b*) Its length and direction is determined by the position and shape of the clear region which, except in *Luzula* and some *Haworthia* species, is contingent on vacuole formation and subject to control by the granular cytoplasm. The propagation of the spatially restricted granular cytoplasm restricts its field by pressing it close to the wall or, as in *Luzula*, to the centre of the pollen mother cell. In *Haworthia* it is presumably governed in part by cytoplasmic granules (which lead to its confinement close to a wall) and by the granular cytoplasm. Thus in most species, absence or incompleteness of vacuole formation lead to the spindle being wrong in direction and length, e.g. *Scilla* the dwarf grains of *Tradescantia* and normal grains following temperature treatment.

7. The generative cell arises next to the wall in the clear, ribose-nucleic acid-free cytoplasm.

8. Yet the nucleus retains a full stock of desoxyribose-nucleic acid, while the vegetative nucleus lying in rich cytoplasm gives up its desoxyribose-nucleic acid.

9. The vegetative cell can divide again either where differentiation of the vegetative and generative cell breaks down, or with gene or chromosome upsets, or where the temperature upsets nucleic acid, protein, or enzyme conditions. The effect may be cumulative as in *Sorghum* with B chromosomes. Extra division does not then occur once, but many times; eventually it leads to death of the pollen grain.

10. Precocious division of the generative nucleus, following upset in protein or enzyme conditions by temperature.

## 10. THE CONTROL OF THE SPINDLE

The control of the spindle length by restriction of the clear zone of cytoplasm, is also seen in the oöcytes of animals, where the spindles are invariably extremely short in relation to cell size. The zone of spindle formation is enclosed by granular cytoplasm much more dense than that of the pollen grain. There seems little doubt that it is responsible for the restriction of spindle length.

The pollen-grain observations throw light on another aspect of spindle formation, *viz.* the hollow spindles seen in white blood precursor cells of mammals (La Cour, 1944), and similarly in the pollen grains of certain *Tulipa* species (Upcott, 1939).

In differentiated blood cells, the cytoplasm of red cells is rich in ribose-nucleic acid and presumably likewise in protein: the spindles are never hollow and the chromosomes are evenly and compactly distributed on the plate. In white cells, however, the cytoplasm is low in ribose-nucleic acid and presumably protein: the spindles are hollow and the chromosomes are arranged on the periphery of the plate. It would seem that hollowness is a sign of weakness, for in pernicious anæmia, and in the mouse as a normal condition, the cytoplasm in some white cells is starved; spindle formation breaks

down and anaphase does not occur. We may therefore be justified in assuming that, in these cells, the granular cytoplasm is deficient in enzymes for conversion of the clear cytoplasm into the *fibrous*, spindle-forming cytoplasm, or perhaps simply that it is more dilute.

What may be the first step in a similar breakdown is to be found in the pollen grains of *Tulipa sylvestris*. Here with extreme degrees of hollowness, chromosomes are left off the plate and are found lying in the granular cytoplasm. The degree of hollow spindle in *Tulipa* is variable, it is emphasised somewhat where the chromosomes have sub-terminal centromeres. It is greatest in the polyploids. No doubt the variability is associated with absence or degree of vacuole formation in unbalanced cells.

## 11. THE SYNCHRONISATION OF MITOSIS

Where thin-walled pollen grains are closely attached in tetrads and even greater associations, as in some orchids, the pollen-grain mitoses are synchronised (Barber, 1942). This is also true under experimental conditions where pollen grains stick together by incompleteness of wall formation. It is true even of chromosome-deficient grains when their cytoplasm is in contact with complementary hyperploid grains (Barber, 1941 ; Sax, 1942). As Barber suggested, nuclear interaction is presumably responsible for the rigidity of synchronisation, just as it is responsible for the survival of adherent deficient grains.

In *Luzula*, Malheiros *et al.* (1947) claim from their observations that synchronisation is always complete and furthermore that it is fairly close between all the grains within an anther. This as we saw earlier is not always the case. How does this difference arise? It may be that in unusual environmental conditions, *e.g.* colder climate, the co-ordination in timing breaks down. Similarly in the dwarf grains, where further division occurs, the two mitoses are not always in step. This may be due to the completeness of the separation of the cytoplasm following the primary division, or to an upset related to the conditions which prompted further division.

## 12. SUMMARY

1. Dwarf pollen grains of obscure origin, are found to occur in variable proportions in a single clone of *Tradescantia bracteata*.

2. Their dwarfness is due to inhibited growth midway in development. The cytoplasm is then deficient in protein and does not accumulate ribose-nucleic acid.

3. They are capable of mitosis and invariably show the normal chromosome number, $n = 6$. Polarity of the mitosis, however, is deranged and differentiation is not attained.

4. Following temperature shock, these abortive grains may undergo further mitosis, to give four equal-sized nuclei, with the cytoplasm equally partitioned between them.

5. In two clones of the same species, an analysis has been made of spindle orientation at the second meiotic division. Cell distribution in both clones proved to be closely similar.

6. The literature on pollen-grain development in Angiosperms is reviewed.

7. An explanation for nuclear differentiation is provided. It is shown that differentiation is dependent on a qualitative difference in the cytoplasm surrounding the two nuclei.

8. The generative cell contains clear cytoplasm with little or no ribose-nucleic acid ; the vegetative cell, cytoplasm rich in ribose-nucleic acid, which is apparently essential to growth and other activities.

9. The inequality in distribution of the cytoplasm is attained by movements of the cytoplasm and nucleus which govern the position, direction, and length of the spindle.

*Acknowledgments.*—The author is indebted to Dr C. D. Darlington, F.R.S., for advice and criticism and to Mr S. Muldal for suggestions concerning the measurement of the angles of the spindles at the 2nd meiotic division.

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PLATE I.—Pollen grain tetrads in *Luzula purpurea*. Acetic alcohol, iron aceto-carmine. Figs. 1-3, × 1800 ; fig. 4, × 950.

FIG. 1.—Pro-metaphase.

FIG. 3.—Nuclear differentiation complete, with the four generative nuclei in the centre.

FIG. 2.—Non-concurrent mitoses.

FIG. 4.—Group of tetrads showing phases of mitosis from metaphase-telophase. The top cell showing the beginning of nuclear differentiation.

PLATE II.—Pollen grains in *Tradescantia bracteata* (clone 20′). Fixation in acetic alcohol. Figs. 1-3 staining with Feulgen ; 4, 7 and 8 in acetic lacmoid ; 5 in toluidine blue ; 6 with Feulgen and counterstaining in Ponceau red, ×1500 except 5 and 6, ×1700.

FIG. 1.—Anaphase in normal size grain.

FIG. 2.—Early telophase with asymmetrical spindle in normal size grain.

FIG. 3.—Anaphase in dwarf grain.

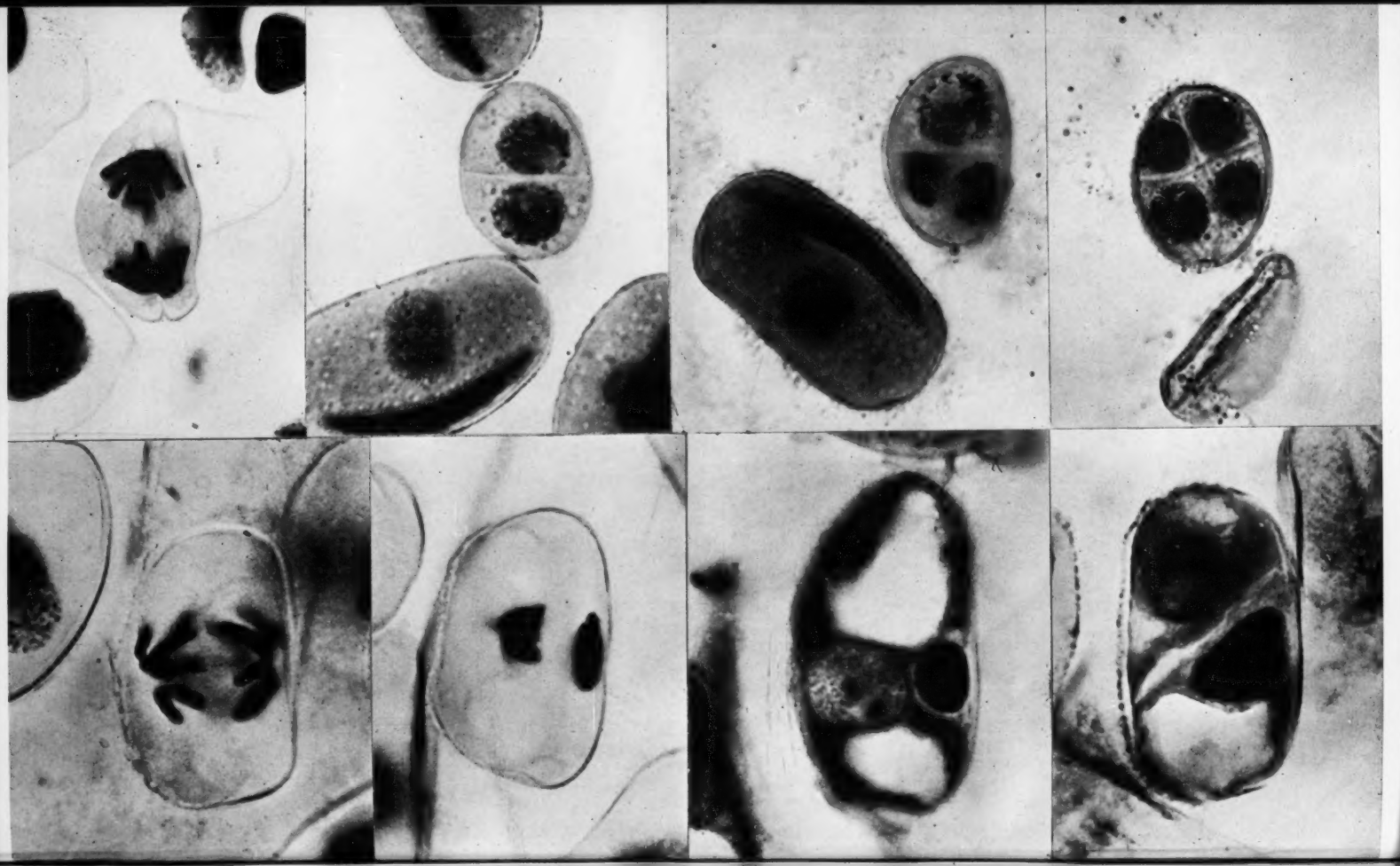
FIG. 4.—Normal and dwarf grains showing undifferentiated nuclei in the dwarf grain.

FIG. 5.—Weakly charged vegetative nucleus in cytoplasm rich in ribose-nucleic acid ; heavily charged generative nucleus in cytoplasm free of ribose-nucleic acid.

FIG. 6.—Partial differentiation of nuclei and cytoplasm.

FIG. 7.—Extra mitosis in dwarf grain induced by temperature shock.

FIG. 8.—Four equal size nuclei in dwarf grain derived from extra mitosis.



# STRUCTURE OF THE INCOMPATIBILITY GENE

## II. INDUCED MUTATION RATE

By D. LEWIS

*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.*

Received 11.iv.49

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## 1. INTRODUCTION

INCOMPATIBILITY genes have been shown to be very suitable for the study of mutations particularly in *Oenothera organensis* and *Prunus avium* (Lewis, 1948). The first object of the work was to test methods for detecting mutants and to find the spontaneous rate in different clones which would be used in X-ray experiments.

The special advantages of the S gene for spontaneous mutation studies have been described previously, and as these apply equally to X-ray work they will not be reiterated.

Some of the results with X-rays reported in this article have been known in other organisms for some time ; this repetition has been necessary, however, in order to find if the new material and methods give a picture of X-ray induced mutation which is consistent with the known data obtained by other methods. Furthermore, as much of the published work on induced mutations has been on lethal genes in *Drosophila*, the publication of similar results with a non-lethal gene and in plants appears to be justified.

In addition some results are new ; these have been revealed only by the special properties of the S gene—properties that facilitate the selection and fine analysis of mutants but that do not distinguish this gene fundamentally from others. Thus it is possible from data obtained with this gene to draw general conclusions.

## 2. MATERIAL AND METHODS

Mutants were detected by two methods, pollen-tube count and seed production, following those already described (*loc. cit.*). The irradiation was given unfiltered at 75 kV and 6 mA with 300 mm. average target distance using a field 150 mm. in diameter, that is, at a mean measured intensity over the field of 110 roentgen units per minute.

With few exceptions temperature was controlled during irradiation to within 1° C. and the material was kept in this temperature for 30 minutes before and 60 minutes after the treatment. Temperature was not otherwise controlled.

The plants treated were *Oenothera organensis* and the sweet cherry, *Prunus avium*. The clones of *Oenothera* and the clonal varieties of *Prunus* and their incompatibility groups after Crane and Brown (1937) are given below :—

*Oenothera organensis*

| | | |
|---|---|---|
| $1^{14}$ / $1^{18}$ S3.6 | $2^{17}$ S4.6 | $1^{2}$ S2.6 |

*Prunus avium*

| Group III, S3.5 | Group V, S3.4 | Group VII, S4.5 |
|---|---|---|
| Bigarreau Napoléon | Bohemian Black | Bradbourne Black |
| Emperor Francis | Late Black Bigarreau | Géante de Hedelfingen |
| Mezel 2 | Turkey Heart | Hooker's Black |
| Ohio Beauty | ... | ... |

In most of the experiments irradiation was given to the flower buds when the pollen mother cells were in meiosis, but buds in earlier and later stages of development and mature pollen were also treated. To determine the stage of development at the time of treatment samples were taken and examined by the aceto-carmine and lacmoid squash methods. The two species differed in the mode of their bud development and thus each required a different experimental procedure. The pollen mother cells in *Prunus* all develop more or less at the same time, thus all the buds on a branch at any one time are, within certain limits, at the same stage of development. These limits were not easy to determine accurately ; pollen mother cells in first and second division could be found in the same sample and second-division stages with tetrads, but it was rare to find pollen mother cells as widely different as metaphase I and tetrads. Thus to compare the effect of treatments at different stages of development several treatments have to be given to different branches over a period of time.

On a single shoot of *Oenothera* there are buds, at any one time, in every stage from minute primordia to open flowers. These are arranged in a regular order on the flowering shoots, one in each leaf axil with the open flower at the base and successively younger

buds towards the top of the shoot. A bud approximately 6 mm. in length, that is, one which is the seventh or eighth above an open flower, is either in, or very near to, the first division of meiosis. Thus to treat buds at different stages of development only one treatment is necessary.

The procedure was to mark buds that were 6 mm. long at the time of irradiation and to use the pollen from the flowers that open each day for 30-40 days after irradiation. The marked buds open into flowers 13-21 days after irradiation according to the temperature conditions. Hence, although the time scale may vary from one experiment to another, the marked buds are strictly comparable and serve as a dividing line to which all other buds can be referred.

### *Calculation of mutation rates*

In all calculations of the number of mutations due to treatment the spontaneous rate has been deducted. The calculation of the number of treated **S** genes tested for mutation is obtained from the formula :—

$$\frac{Nk}{2^n}$$

Where N = the number of flowers pollinated
$k$ = the number of pollen grains on a stigma
$n$ = the number of gene reproductions between the stage treated and the gene reproduction immediately preceding pollen grain mitosis.

## 3. RESULTS

### (*a*) *Effect of dosage*

No large scale experiment was made to establish the relationship between mutation and the X-ray dose, but tests to find the best dose for other experiments gave some data, which, although meagre, at least do not conflict with the direct relationship that has been found in other gene mutation studies. Tests made in *Prunus*, group V, S3.4, are summarised in table 1 and fig. 1.

TABLE 1

*Fruit set from X-ray doses given at early prophase after incompatible pollination in* Prunus *group V*

| Dose *r* units | Temperature | Flowers pollinated | Fruits set | Fruits per 100 flowers due to treatment * |
|---|---|---|---|---|
| 90 | 15° | 351 | 1 | 0·03 |
| 200 | 15° | 405 | 4 | 0·63 |
| 300 | 15° | 948 | 8 | 0·59 |
| 450 | 15° | 1181 | 16 | 1·09 |
| 600 | 18° | 742 | 21 | 2·60 |
| 800 | 18° | 885 | 21 | 2·12 |

* In this column a value of 0·25, which is the fruit set due to spontaneous mutation has been subtracted.

### (b) Temperature during irradiation

Although Timoféeff Ressovsky and Zimmer (1939) with sex-linked lethals and Muller (1940) with sex-linked lethals and II and III chromosome translocations in *Drosophila* and Stadler (1930) with dormant and germinating barley seeds found no effect of temperature

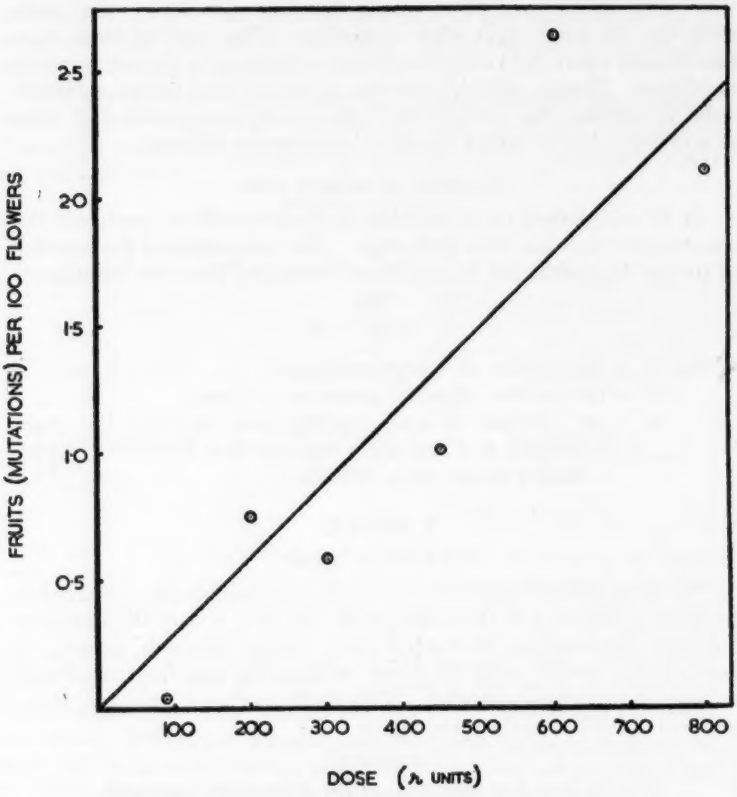


FIG. 1.—Relationship between mutation frequency and X-ray dose in *Prunus avium*.

during irradiation it was thought necessary to check this for three reasons :—(1) The very different states of the nuclei in the materials used—pollen mother cells of *Prunus* and *Oenothera* as compared with ripe sperm of *Drosophila* or with somatic tissue of barley seeds. (2) The difference in the selective sieves through which the mutants have to pass—S gene mutations induced in pollen mother cells must be viable in the haploid gametophyte, while recessive lethals in *Drosophila* and mutants in barley seeds are subjected to a less rigorous selection

which allows gross chromosomal changes to pass. (3) The positive effect of temperature on chromatid reunion and sister reunion in *Tradescantia* pollen grains (Darlington and La Cour, 1945).

The results in *Prunus* show that temperature has an effect both on the percentage of fruits set and on seeds produced when the irradiation is given in the early prophase of meiosis (table 2). This effect is highly significant in group VII cherries and, although in group III the results are not significant the difference is in the same direction.

TABLE 2

*Effect of temperature during and 1 hour after irradiation in* Prunus avium ; *results above and tests of significance below. Dose, 600* r.

| Group | Genotype | Temperature °C. | Stage at irradiation | Flowers | Fruit set per cent. | Seeds per cent. |
|---|---|---|---|---|---|---|
| III | $S_{3}$-$S_{5}$ | 9 | Early prophase | 1732 | *0·4* | 0·18 |
| ,, | $S_{3}$-$S_{5}$ | 24 | ,, ,, | 960 | *0·6* | 0·48 |
| ,, | $S_{3}$-$S_{5}$ | 10 | Metaphase | 2151 | *1·5* | 0·97 |
| ,, | $S_{3}$-$S_{5}$ | 24 | ,, | 1995 | *1·6* | 0·80 |
| VII | $S_{4}$-$S_{5}$ | 10 | Early prophase | 1619 | *1·0* | 0·32 |
| ,, | $S_{4}$-$S_{5}$ | 24 | ,, ,, | 1452 | *2·1* | 0·83 |

| Variable | Constant | $\chi^2$ | $p$ |
|---|---|---|---|
| Temperature | Prophase group III | 0·278 | ·6 |
| ,, | ,, ,, VII | 7·233 | <·01 |
| ,, | Metaphase ,, III | 0·040 | ·9 |
| Stage | 10° ,, III | 20·165 | <·01 |
| ,, | 24° ,, III | 4·661 | ·03 |

Controls using a high temperature without X-radiation, were provided from an experiment designed to detect any effect of temperature on spontaneous mutation. Group III trees of *Prunus* were kept at 27° C. for three different periods :—(1) 4 days from the resting stage to metaphase of meiosis, (2) 13 hours from zygotene to the end of pachytene and (3) 13 hours from diakenesis to metaphase. No effect of these treatments on the spontaneous mutation rate could be found ; the combined data are given below :—

| | | Flowers | Fruit set |
|---|---|---|---|
| Treatments, 1, 2 and 3 | 27° C. . . | 3054 | *0·16* per cent. |
| | 12-13° C. . . | 6428 | *0·13* ,, |

For a fifteen degree rise in temperature the mutation rate of the S gene under X-radiation is approximately doubled.

In both groups the effect appears to be greater when measured as " seed set " than as " fruit set " and this is probably an important difference because the seeds represent only mutations of the S gene that are fully viable in the male gametophyte and are not lethal in

the embryo, while the fruits include mutations that are dominant lethals in the embryo as well.

During irradiation given at a stage which is estimated by examination to be metaphase I—or at least with certainty when the chromosomes are spiralised and condensed—temperature has no effect.

Results of a temperature test in *Oenothera* show an effect similar to that in *Prunus*, table 3 ; again a rise of 14° C. during irradiation produces a doubling of the mutation rate. It was not possible with these data in *Oenothera* to distinguish sharply between mutations occurring under different states of the chromosomes, as the treatments had to be given to buds in every condition and the total number tested was not large enough to be able to separate the mutants in respect to the stage of treatment.

TABLE 3

*Effect of temperature during and 1 hour after irradiation in* Oenothera organensis. *Dose 1000* r.

| Clone | Genotype | Temperature | Flowers | Pollen tubes per 100 flowers | $\chi^2$ | $p$ |
|---|---|---|---|---|---|---|
| $1^2$ | S2.6 | 18 | 74 | 4·1 | 1·216 | ·2 |
| | S2.6 | *32* | *108* | *7·9* | | |
| $1^{18}$ | S3.6 | 18 | 121 | 6·9 | 2·406 | ·1 |
| | S3.6 | *32* | *35* | *15·8* | | |

With the high dose employed in this experiment, buds irradiated late in meiosis tend to fall-off prematurely, and therefore mutants induced during this stage are underestimated in the sample. Thus the majority of mutants in these samples represent changes occurring early in prophase of meiosis.

The *Oenothera* results are not significant in themselves but taken with the *Prunus* results there is overwhelming evidence for the temperature effect.

### (c) *Sensitivity and the nuclear cycle*

The data on *Prunus avium* given in table 2—assembled primarily to show the effect of temperature—hint at a different sensitivity to X-rays between prophase and metaphase of meiosis.

Thus at 9-10° C. the percentage of fruit set with irradiation at metaphase is more than three times that from comparable prophase treatments ; the same comparison based on seed set shows more than a fourfold difference. At 24° these differences are smaller, nevertheless at both temperatures the differences are significant as shown by the $\chi^2$ in table 2.

On the most acceptable view of the meiotic chromosomes that chromosome division occurs at pachytene, and on the assumption that the gene reproduces at about the same stage, much of the apparent difference in sensitivity to X-rays is accounted for. To make a

comparison of sensitivity we must, therefore, apply a correction factor of 2, either by multiplying the number of "prophase" mutants, or dividing the number of "metaphase" mutants, by two. Values corrected by halving the number of metaphase mutants give a $\chi^2$ of 1·558 ($p = ·2$) at 9° and 0·165 ($p = ·6$) at 24°. Thus the difference at 24° has largely disappeared, while at 9°, although the difference is not now significant, it suggests a real effect.

TABLE 4

*Mutations produced by 600* r *units at 20° C. given to buds of* Oenothera organensis $1^{14}$, S3.6 *showing the relationship between stage of treatment and the number of mutants*

| | | Days after irradiation | Number of flowers | Compatible pollen-tubes (mutations) | Induced mutations per $10^8$ S genes * |
|---|---|---|---|---|---|
| P.G. | | 3 | 19 | 1 | |
| | | 4 | 8 | ... | |
| | | 5 | 9 | ... | |
| | | 6 | 12 | 1 | |
| | | 7 | 8 | ... | |
| | | 8 | 36 | ... | |
| | | 9 | 26 | ... | |
| | | 10 | 23 | 1 | |
| | | 11 | 38 | ... | |
| | | 12 | 44 | 1, 1 | |
| | | 13 | 34 | ... | |
| Meiosis | Meta-phase | 14 | 44 | 1, 1 | |
| | | 15 | 31 | 1, 1, 1, 1 | |
| | | 16 | 97 | 1, 1, 1, 1, 1, 1, 1, 1, 1, 1 | 17·8 |
| | | 17 | 60 | 1, 1, 1, 1 | |
| | | 18 | 60 | 1, 1 | 5·5 |
| | | 19 | 105 | 1, 1, 1, 1 | |
| | | 20 | 76 | 2, 2, 1, 1, 1 | |
| | | 21 | 94 | 4, 2, 2, 1, 1, 1 | 21·0 |
| | | 22 | 94 | 2, 1, 1, 1 | |
| | | 23 | 35 | ... | |
| | | 24 | 20 | 16 | |
| | | 25 | 46 | 1, 1 | |
| | | 26 | 44 | ... | |
| | | 27 | 71 | 4, 2, 1, 1 | |
| | | 28 | 18 | ... | |
| Mitosis | | 29 | 60 | 1, 1 | |
| | | 30 | 42 | ... | |
| | | 31 | 47 | 1 | |
| | | 32 | 36 | 6, 3 | |
| | | 33 | 54 | 9, 1 | |
| | | 34 | 37 | ... | |
| | | 35 | 79 | 1, 1 | |
| | | 36 | 24 | ... | |
| | | 37 | 23 | 2 | |
| | | 38 | 16 | ... | |
| | | 39 | 46 | 1, 1 | |

* Spontaneous mutations (1·7 per $10^7$ S genes) have been deducted. It is not possible to calculate the mutation rate based on the number of genes irradiated later than the 22nd day after treatment, because the mitotic divisions occur in rapid succession thus making it impossible to estimate the number of genes treated.

Further evidence on this point comes from *Oenothera*. The data given in table 4 are the combined results of two irradiations, one given in 1946, and the other in 1947. The distribution of the mutants

with time *after* irradiation, and therefore, with the stage of development at irradiation, was very similar in the two treatments; thus they have been combined for ease of presentation.

There are three points emerging from this experiment :—

(i) The great increase in the percentage of styles found with a single compatible (mutant) pollen tube from pollen irradiated late in meiosis.

(ii) The appearance of two or more compatible pollen tubes in a single style from pollen irradiated 5 days before metaphase of meiosis. This is followed within 24 hours by groups of four compatible pollen tubes.

(iii) A tendency to a low percentage of mutants occurring after the high number at metaphase and before the appearance of " double " mutants.

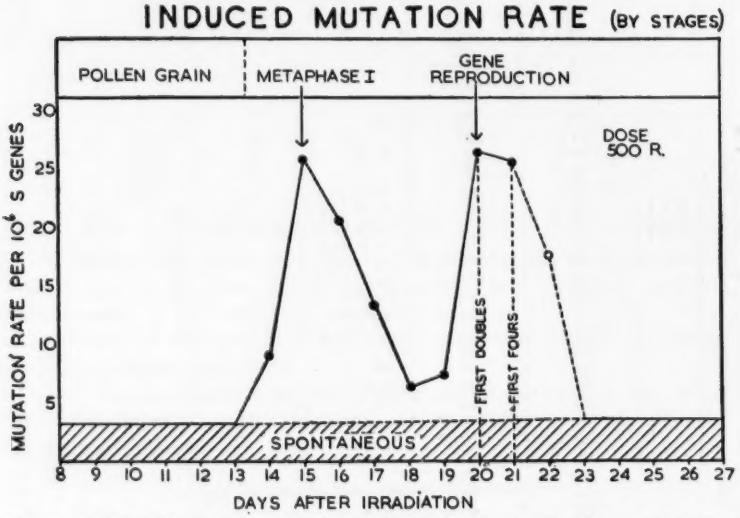


FIG. 2.—Relationship between nuclear stage irradiated and the sensitivity to X-rays as measured by the induced mutation of the **S** gene in *Oenothera organensis* by the compatible pollen tube method.

Double mutants are the result of irradiation before the **S** gene has divided. Therefore, to make a comparison of the sensitivity at different stages, the number of such mutants must be multiplied by two. This and a correction for the spontaneous rate has been made in fig. 2 which clearly shows a highly sensitive period when the chromosomes are condensed in late meiosis with metaphase I at the peak, preceded by a less sensitive stage, and again by a more sensitive stage when double mutants appear.

These results confirm those in *Prunus* and together they suggest that it is a general rule for gene sensitivity to X-rays to vary during

the nuclear cycle. There are, however, certain aspects of the results in the two species that can only be reconciled by assuming that the gene divides from the point of view of X-ray mutability before pachytene. For, if the period of low sensitivity corresponds to the same stage of the nuclear cycle in the two species, this stage being the beginning of prophase in *Prunus*, then the absence of double mutants in *Oenothera* means that the gene has divided before this early stage.

Another and quite different aspect of the induction of mutants by X-rays at different stages of development is found in the mature pollen. Doses up to 4000 *r* have been given to mature *Oenothera* pollen without inducing mutations. This is due simply to the fact that the character, mating reaction of the pollen, has been laid down before this stage. In a similar way large doses given to mature pollen have no effect on germination (Poddubnaja-Arnoldi, 1936; Newcombe, 1942).

### (*d*) *The mutation constant*

To be able to compare the effect of a comparable dose of X-rays in *Prunus* and *Oenothera*, the mutation constants (the probability $\alpha$ that a gene should mutate for a dose of one roentgen unit) are given

TABLE 5

*Mutation constants calculated for 1 r unit of X-radiation. ("Fruit," "Seed," and "Pollen tube" refer to the method of scoring. The rates calculated from seed set are lower than from fruit set owing to zygotic lethals.)*

| Genotype | Temperature °C. | Stage at irradiation | Mutation constants $\times 10^{-8}$ | | |
|---|---|---|---|---|---|
| | | | Pollen tube | Fruits | Seed |
| *Prunus avium* | | | | | |
| $S_{3.5}$ | 9 | Early prophase | ... | 1·4 | 0·7 |
| | 24 | ,, ,, | ... | 1·9 | 1·4 |
| $S_{4.5}$ | 10 | Metaphase | ... | 2·3 | 1·5 |
| | 24 | ,, | ... | 2·4 | 1·3 |
| | 10 | Early prophase | ... | 3·0 | 1·1 |
| | 24 | ,, ,, | ... | 6·8 | 2·7 |
| *Oenothera organensis* | | | | | |
| $1^{2}$ $S_{2.6}$ | 18 | All stages * | 1·5 | ... | ... |
| | 32 | ,, ,, | 3·1 | ... | ... |
| $1^{18}$ $S_{3.6}$ | 18 | ,, ,, | 2·8 | ... | ... |
| | 32 | ,, ,, | 6·8 | ... | ... |
| $1^{14}$ $S_{3.6}$ | 20 | Early prophase | 1·0 | ... | ... |
| | 20 | Metaphase | 2·9 | ... | ... |

* Calculated from the number of pollen grains and not from the number of treated S genes.

in table 5. The constants for the prophase treatments in *Prunus* have been derived from figures corrected by a factor of 2 based on the

assumption that the gene had not reproduced at this stage. If, as was suggested previously the gene had reproduced before this, these constants are twice too large. By not applying this correction factor in *Prunus*, the comparison between metaphase and prophase sensitivities would agree even more closely in the two species.

It is clear from the table that the constants differ according to the clone, the stage of nuclear cycle and the temperature at irradiation, and the range of variation due to these causes is similar in the two species.

### (e) *Qualitative tests on mutants*

Induced mutations of the incompatibility gene obtained as seeds in *Prunus avium*, have produced progeny but it will be several years before they flower and can be tested qualitatively. In the meantime similar mutants in *Oenothera organensis* have flowered and have been tested.

From 19 flowers of clone $1^{14}$ (S3.6) that were pollinated in 1945 with pollen from anthers of clone $1^{18}$ (S3.6), irradiated with 500 *r* units, 37 days previously, one capsule was obtained which contained 36 seeds. Thirty-four plants, all of normal vigour, were produced and these were all fully self-compatible. The results of selfing tests on a selection of the plants is given in table 6. And from pollen-tube

TABLE 6

*Results of self-pollination of self-compatible* Oenothera *plants,* S*3.6′ and* S*6.6′ from an X-ray mutation, compared with compatible pollination of normal plants*

| | Different plants | Flowers | Capsules | Mean seeds per capsule |
|---|---|---|---|---|
| S3.6′ selfed . . | 9 | 10 | 10 | 278 |
| S6.6′ selfed . . | 11 | 12 | 12 | 264 |
| Normal crossed . | 4 | 17 | 17 | 242 |

growth studies and records of capsules set on all the plants after self-pollination no difference whatever could be found between their behaviour on selfing and the behaviour of normal plants after compatible cross-pollination.

Crosses between these self-compatible plants and test plants of known S genotypes revealed two different groups (table 7). Group I plants as females inhibited all the pollen of the original plant S3.6, while the other group inhibited only half of the pollen of the same plant. In four crosses with S2.3 and S2.6 plants the two groups behave differently.

There is only one interpretation of these results, *viz.* that in the irradiated S3.6 plant an S 6 allele mutated to what may be symbolised as S6′. When pollen carrying S6′ was put on an S3.6 plant half the progeny was S3.6′ and half S6.6′; these are the two groups I and II respectively.

All the group II plants were intercrossed in 56 different combinations and in all cases, as expected, half the pollen was compatible and half incompatible.

TABLE 7

*Incompatibility reactions of* Oenothera *plants with the mutant allele* S6′ *as follows:* ++, *all pollen compatible;* +−, *compatible and incompatible pollen in a* 1 : 1 *ratio;* −−, *all pollen incompatible*

| Group I S3.6′ | | | Group II S6.6′ | | |
|---|---|---|---|---|---|
| S3.6′ selfed or intercrossed | + | − | S6.6′ selfed or intercrossed | + | − |
| S3.6 × S3.6′ | + | − | S3.6 × S6.6′ | + | − |
| S3.6′ × S3.6 | − | − | S6.6′ × S3.6 | + | − |
| S2.6 × S3.6′ | + | + | S2.6 × S6.6′ | + | − |
| S2.3 × S3.6′ | + | − | S2.3 × S6.6′ | + | + |
| S3.6′ × S2.6 | + | − | S6.6′ × S2.6 | + | − |
| S3.6′ × S2.3 | + | − | S6.6′ × S2.3 | + | + |

It will be seen from table 7 that a novel feature of this mutant allele (S6′) is that, despite its complete lack of action in the pollen grain, it operates fully in the style to inhibit S6 pollen (fig. 3).

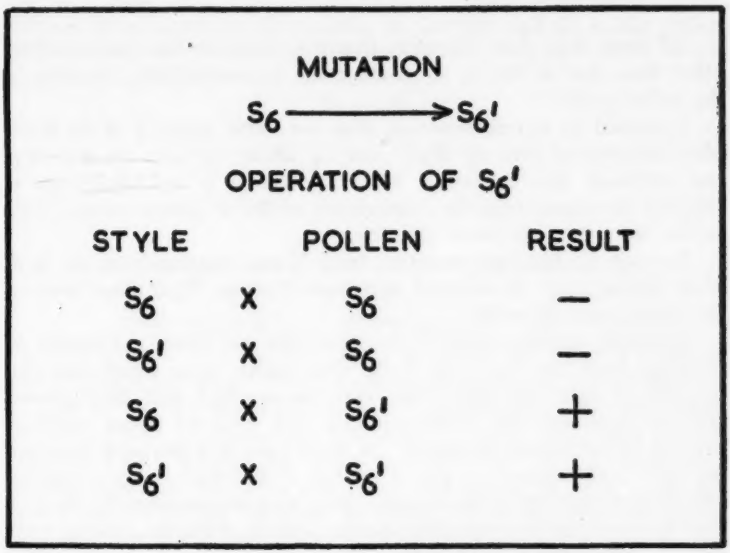


FIG. 3.—The operation of an X-ray induced mutant allele, S6′.

Progeny raised from a plant from each self-compatible group were tested with the relevant test plants and each segregated into two classes. The group I plants (S3.6′) produced S3.6′ and S6.6′ in approximately

equal numbers. The group II (S6.6′) plant produced S6.6′ and S6′.6′ plants ; both results confirm the nature of the mutant allele.

On testing the S6′.6′ plants further it was found that when used as females they were fully incompatible with S6 pollen. Thus the S6′ allele is fully operative in repelling S6 pollen even when not accompanied by a normal allele such as S6 or $S_3$.

More detailed tests were made to find if the S6′ allele had any other effect, a deleterious effect for example which is often associated with a minute deficiency. The results were as follows :—

(i) Both the pollen-germination and pollen-tube growth of S6′ were equal to those of compatible pollinations with pollen carrying normal alleles.

(ii) In a cross-pollination between $S2.4 \times S3.6'$, that is, when S6′ pollen competes on equal terms with $S_3$, the progeny segregated into 7 self-incompatibles and 7 self-compatibles, showing that S6′ pollen is at no serious disadvantage when compared with $S_3$.

(iii) Plants carrying one or two S6′ alleles were in no way less vigorous than those with normal alleles.

(iv) Styles which were S3.6′ inhibited S6 pollen just as strongly as S3.6 styles.

All these tests show therefore that the mutation has had no effect other than that of failing to produce the incompatibility reaction in the pollen grain.

It should be stated, however, that the selfed progeny of an S3.6′ plant segregated into 23 S3.6′ and 14 S6′.6′ ($\chi^2 = 2{\cdot}18$, $p = {\cdot}15$) and although this deviation from expectation is not significant it suggests that there may be a deficiency of S6′.6′ plants which could be due to embryos of lower viability.

In 1946 an induced mutation from X-ray treatment on an S2.6 plant and in 1947 six induced mutations from an S3.6 plant were of the same type—S6→S6′.

However, another type of mutation was also found. Families of seedlings from six mutations from S3.6 plants were tested and the progeny of each segregated into two classes, S3.6 and S6.6 plants. Neither class was fully self-compatible but each set a few seeds at the end of the flowering season. In these cases it is probable that the S6 allele had mutated to give a weaker allele which did not operate efficiently at the end of the season. This view is supported by the fact that in other self-incompatible species such as *Nicotiana sanderæ*, end-season compatibility is known. The low temperature prevailing at this season also contributes to weakening the incompatibility reaction.

It is of interest that these mutants arose after pollinations made on end-of-season plants grown out-of-doors. In other words the rigorous selective mechanism of incompatibility was relaxed allowing small

mutations that have only a slight effect on incompatibility to get through and produce seeds. Hence the kind of mutants found will depend largely on the selective mechanism operating.

### (*f*) *Tests for crossing-over within the* S *gene*

The previous results have shown that the S gene behaves towards X-rays as if it were of two parts, one part operating only in the pollen and the other in the style and possibly in the pollen also. It seemed desirable, therefore, to test for crossing-over between the two parts. But before such tests could be planned it was necessary to define clearly the activity of the parts. We know that the S6′ allele (the X-ray mutant which is inactive in the pollen) is fully active in its original reaction in the style. This means that the stylar part of the S gene is specifically different for each allele. We do not know, however, if the pollen-active part is specific for each allele, or if it is non-specific and common to all alleles, merely extending to the pollen grain the activity of the stylar part.

If it is non-specific then crossing-over between say $S_3$ and S6′ would give $S_3'$ and S6, which would restore the mutant S6′ allele to its original state before mutation. If, on the other hand, the pollen part is specific then crossing-over will produce an entirely new allele, $S6^3$ which will have an S6 reaction in the style and $S_3$ in the pollen. The first test was based on the assumption that the pollen active part is non-specific :—

$S_{2.3} \times S_{3.6}'$

| Non-crossovers | Crossovers | |
|---|---|---|
| $S_{2.6}'$ $S_{3.6}'$ | $S_{2.3}'$ $S_{3.3}'$ | $S_{2.6}$ $S_{3.6}$ |
| Self-compatible | Self-compatible | *Self-incompatible* |

Thus any self-incompatible plants would be the result of crossing-over within the S gene. From 149 plants tested none were found.

The second test was based on the assumption that the pollen-active part is specific. To make this test clear we must represent a normal allele as $S3^3$, 3 referring to the stylar reaction and $^3$ to the pollen reaction, because both are specific to the particular allele. If S6′ crosses over with $S3^3$ then an $S6^3$ allele would be produced which would have a 6 reaction in the style and a $^3$ reaction in the pollen. The test cross is then :—

$S2^2$ $S3^3 \times S3^3$ S6′

crossover

$S2^2$ $S3'$ (Exceptional plant from crossing-over)

The exceptional plant should be self-compatible, and incompatible as a female to $S2^2$ $S3^3$ plants. None of these were found: crossing-over had not occurred.

If the two parts are both specific, when crossing-over occurs between two normal alleles, *e.g.* $S3^3$ and $S6^6$, a plant carrying such a composite allele, $S3^6$ or $S6^3$ would be self-compatible. Out of large numbers grown both at Merton and by Sterling Emerson in Pasadena, no self-compatible plants have been found except as a result of X-radiation. Crossing-over except as an extreme rarity can therefore be excluded.

## 4. DISCUSSION

### (*a*) *Nature of* S *gene mutants: temperature effect*

None of the mutations of the S gene induced by X-rays have any measurable effect, even when homozygous, on the viability and fertility of the diploid plant, or on the haploid male or female gametophytes. This contrasts sharply with the results obtained by Stadler (1941) who found, with endosperm genes in maize, that all the X-ray induced mutants had very marked subsidiary effects on viability and fertility. He interpreted this as due to the visible endosperm mutations being associated with minute chromosomal deficiencies.

The explanation of the difference between the two results lies in the different selection that follows the X-ray treatment in the two cases.

The S gene mutants, from irradiations given at or before meiosis, have to be viable in the male haploid gametophyte before they can be detected. All haplo-lethals are therefore excluded. The maize mutants, on the other hand, are from treatments given to mature pollen, and since mutants produced at this stage are not likely to be expressed until after fertilisation, lethal mutations of all types will not be selected against.

This rigorous selection of the S mutants against lethality may have an important bearing upon a comparison with other gene mutation studies of effects such as temperature during irradiation.

Published accounts of the effects of temperature on the frequency of X-ray induced mutation in *Drosophila* are conflicting. Timoféeff-Ressovsky and Zimmer (1939) and Muller (1940) found no effect of temperature differences ranging from 3-37° C. on sex-linked lethals induced by 1200-3000 *r* units. On the other hand King (1947) found the significant effect that the frequency of sex-linked lethals induced by doses ranging from 600-3600 *r* units was 2·3 times higher at 0° than at 25° C.

The positive effect of high temperature on increasing the number of induced viable S mutations in *Prunus* and *Oenothera* is apparently at variance with all these. However, if we assume a reduction by high temperature of the number of lethals mutations—associated probably with gross chromosomal changes—as found by King in *Drosophila* there would be a secondary effect of increasing the number

of fully viable mutations because there would be less loss due to associated lethals.

The fact that the temperature effect on the **S** gene mutation does not operate when the chromosomes are fully condensed and charged with nucleic acid suggests that chromosome restitution is involved. If breaks that have restituted are less likely to be lethal than those which have not, then more restitution would lead to a higher viable **S** mutation under the selective conditions of the method. Since fully charged chromosomes are held together with nucleic acid and do not break it is to be expected that temperature under such conditions would have no effect. This would also explain the greater production of mutations when irradiation is at metaphase.

The whole problem of temperature and induced mutations, however, is not sufficiently known to be able to generalise and it is clear that much more work is necessary before a solution can be found.

### (*b*) *Divisibility of the gene*

The **S** gene has been shown by the present experiments to consist of at least two parts, one part active in the pollen and the other in the style. Before this was known it had been shown, by examining the effect of this gene in diploid pollen of induced tetraploids that the different **S** alleles, while they do not interact in the style, show dominance and competition in the pollen (Lewis, 1947). This differential property as regards interaction in different tissues corroborates the theory that this gene is made up of 2 units.

The double structure of the **S** gene raises the general question of the constitution of the gene, but as this has been considered in detail by Raffel and Muller (1940) as a result of work on the *Scute* gene in *Drosophila* it will not be discussed except in so far as the present and recent published results extend it.

For a full discussion of the possible nature of multiple alleles, that is, whether they are variants of a single unit or rearrangements among a number of linked units, see Silow and Yu (1942).

For mutation to occur in one part of the **S** gene, without in any way affecting the other part, is exactly similar to the spontaneous independent mutation of the two parts of the **R** gene in maize (Stadler, 1946). Thus, we are led to the inescapable conclusion that these genes are composite structures of parts which can reproduce without the presence of the other and hence the parts have the essential properties of genes. We have therefore the picture of a gene as a mechanical and functional unit which is equal to two mutational units. These units are held together in such a way at meiosis that no crossing-over takes place between them.

One of the most easily detected suppressors of crossing-over is a small inversion, and this could account for the absence of crossing-over between the units of two alleles, for example within the two composite incompatibility alleles in heterostyled plants (Ernst, 1936). In *Primula*

there are two normal alleles each made up of three units, one controls anther height, one style length, one pollen size and incompatibility. But an inversion cannot prevent crossing-over between every possible pair of the hundreds of different incompatibility alleles that are present in homomorphic species such as *Oenothera organensis*.

## 5. SUMMARY

1. X-radiation was applied to *Prunus avium* and *Oenothera organensis*, when the pollen mother cells were in meiosis or at earlier stages. It increased the number of seeds or of compatible pollen tubes produced, according to the technique—after incompatible pollination. This was due to induced mutation of the incompatibility (S) gene. Treatments given to mature pollen had no immediate effect because the incompatibility reaction in the pollen had already been laid down.

2. High temperature at the time of irradiation when the treatments were given to nuclei in early prophase of meiosis increased the number of S mutations, but had no effect on nuclei treated in metaphase.

3. Treatments given at first metaphase produced 2-3 times as many mutants as the same treatment given at early prophase.

4. Groups of two compatible pollen tubes arising from one anther in *Oenothera* were the result of a mutation in an S gene which had one division to make before reaching the young pollen-grain stage. These double mutants appear in treatments that were given 5 days before metaphase I, but not in later treatments. This showed that the S gene divides mutationally long before pachytene when the chromosomes split.

5. Out of fourteen different X-ray mutants in *Oenothera*, eight of these (S6→S6′) gave complete self-compatibility to the plant by the failure of the S6′ allele to operate in the pollen; in the style, however, the mutant allele was fully operative in its original way (S6). The other six mutants were only partially self-compatible at the end of the flowering season. These partial mutants, which may be the result of smaller gene disturbances, were obtained by the use of a less rigorous selective mechanism.

6. None of the induced mutant alleles had any effect, even when homozygous on the vigour or fertility of the plant or of the male or female gametophytes.

7. The S gene is shown to be of at least two parts, one operating in the pollen and the other in the style, between which crossing-over is excluded.

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# A POSSIBLE INTERPRETATION OF THE APPARENT INTERFERENCE ACROSS THE CENTROMERE FOUND BY CALLAN AND MONTALENTI IN *CULEX PIPIENS*

By A. R. G. OWEN
*Department of Genetics, Cambridge*

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## 1. INTRODUCTORY

CALLAN and Montalenti (1947), in a study of the mosquito *Culex pipiens*, found evidence of an apparent chiasma interference operating between the arms of bivalents at meiosis. They concluded from counting of types of bivalents that chiasma interference functions across the centromere in chromosomes of this organism. It is possible, however, to interpret their chiasma statistics in a rather different way. It is possible to get a good fit to the distribution of bivalent types observed by Callan and Montalenti, on the assumption that chiasma interference (in the usual sense) does not act through or across the centromere. This can be done if we assume that the first chiasma to be formed in a chromosome pair, being a necessity for bivalent formation (and therefore an essential prerequisite for subsequent chiasmata), is not on an equal footing with the chiasmata formed later. It is shown here that this hypothesis of a *primary* chiasma gives a legitimate explanation of the observations of Callan and Montalenti on both *Culex pipiens* and *Theobaldia longiareolata* Macq.

## 2. THE OBSERVATIONS OF CALLAN AND MONTALENTI

Three pairs of chromosomes were found in *Culex*. Two pairs (M) are equal in their apparent physical lengths, and are about $1\frac{1}{2}$ times as long as the third pair (*m*). All six chromosomes have median centromeres. Though the physical length of arm seems comparable with that of other mosquito species (e.g. *Theobaldia*), the *Culex* chromosomes are " short " in respect of chiasma formation, since :—

(i) Mean chiasma frequency per arm of bivalent is less than two-thirds.

(ii) The proportion of bivalents with more than one chiasma on either arm is very small (1·74 per cent.).

Leaving these latter bivalents out of account, Callan and Montalenti counted the numbers of bivalents (both M and *m*) in the three classes O/O, O/X, X/X. (O/O denotes the univalent pairs, O/X denotes bivalents with one chiasma, and X/X denotes bivalents

with one chiasma in each arm.) The percentage in these three classes (copied from their paper) are given in table 1.

For each specimen a smaller proportion of X/X bivalents was found than would be expected on the assumptions of statistical independence between arms, and an equal initial probability of a chiasma appearing in either arm.

For four of the specimens, the bivalents (M chromosomes only) which showed at least one chiasma (*i.e.* types O/X, X/X) were further classified according to the scheme O/P, O/D, P/P, P/D, D/D. (Here O denotes absence of chiasmata from one arm, P the presence of a chiasma in a proximal region of an arm, D the presence of a distal chiasma.) Table 2 is taken from the paper of Callan and Montalenti and shows the percentages in each of these five classes.

Callan and Montalenti interpreted these figures as implying :—

(i) The presence of a chiasma on one arm tends to inhibit formation of a chiasma on the other arm.

(ii) A proximal chiasma on one arm has a greater inhibitory effect on the chiasma in the other arm than has a distal chiasma.

(iii) A chiasma on either arm tends to "repel" a chiasma formed on the other.

That is to say, the observations were taken to imply that in *Culex* the centromere is not effective as an insulator between arms in respect of chiasma interference. *Culex* differs, therefore, from *Theobaldia*, in which the observed frequencies of bivalent types were in accordance with those to be expected if the arms were independent.

## 3. INTERPRETATION IN TERMS OF AN OBLIGATORY CHIASMA

The conclusions derived from their data by Callan and Montalenti depend on the assumption that the two chiasmata observed on the same bivalent are on entirely the same footing : *i.e.* each has the same initial probability as the other, but when formed diminishes the probability (conditional on its own presence) of the other being formed, to a value less than the initial probability of formation of the latter, had the former not been established.

If this be granted, then their conclusions follow.

However, it has sometimes been suggested that the first chiasma to be formed has a rather different status from its successors. The first chiasma, on this view, may be called *obligatory* or *primary* since it is essential for proper synapsis and disjunction to take place. If it be formed, then other chiasmata may follow on the same bivalent. If it be not formed, a bivalent is not constituted by the pair of chromosomes concerned, and further chiasmata will not be established.

On this view we might expect that, as a result of evolutionary modification, the initial probability "$p$" that this first, obligatory,

chiasma be formed on the chromosome pair would have achieved a high value ; perhaps in the range 0·90-1·0. And indeed the figures in table 1 agree well with this line of thought. They imply that the probability that at least one chiasma be set up on a given pair is precisely in this range.

Proceeding with this argument, it does not seem necessary to assume that when the primary chiasma has been established, the probability $q$, conditional on that obligatory chiasma, of a second chiasma appearing in the other arm should be the same as the initial probability of the first one being established. If $q < p$ then it is easily seen that there will be apparent interference between chiasmata on different arms, for it is not possible by inspection to distinguish between the primary chiasma and the secondary one when both are present. In this interpretation of the data, the primary chiasma can hardly be said to interfere with the establishment of the secondary because it is a necessary prerequisite for the existence of the latter.

To argue more precisely, let it be assumed :—

(i) The arms are identical in length and properties.

(ii) In all circumstances chiasma interference operates within each arm to such an extent that the probability of two chiasmata in one arm is negligible.

(iii) A first chiasma is essential for bivalent formation and has the probabilities $p$, $1-p$ of being or not being formed. Suppose that it has the probabilities $pp_1$, $pp_2$ of occurring proximally or distally.

Under these conditions, when a primary chiasma has been formed in either arm, then by (i) and (ii), a secondary chiasma can be formed only in the other arm. Suppose :—

(iv) The secondary chiasma occurs or does not occur with probabilities $q$, $1-q$ *independent of the position in its own arm of the primary chiasma*, and that it may occur proximally or distally with respective probabilities $q_1$, $q_2$ *independent of the position of the first chiasma.*

Clearly $p_1+p_2 = 1$, $q_1+q_2 = q$. The classes O/O, O/X, X/X have relative frequencies $1-p$, $p(1-q)$, $pq$. The classes O/P, O/D, P/P, P/D, D/D occur with relative frequencies $p_1(1-q_1-q_2)$, $p_2(1-q_1-q_2)$, $p_1q_1$, $p_2q_1+p_1q_2$, $p_2q_2$. If $a$, $2b$, $c$ are the observed numbers in the classes O/O, O/X, X/X the maximum likelihood estimators of $p$ and $q$ are simply the ratios

$$\hat{p} = \frac{a+2b}{a+2b+c}, \quad \hat{q} = \frac{a}{a+2b}.$$

These are given in table 1, being calculated from the percentage values $a/(a+2b+c)$, etc., given by Callan and Montalenti. These estimated values, of course, give an exact fit to their percentage data.

A more severe test of the present hypothesis consists in finding values $p_1$, $p_2$, $q_1$, $q_2$ which will give a good fit when used to reconstruct the percentages in table 2. This can be done quite successfully.

TABLE 1

| Specimen no. | O/O | O/X | X/X | Estimated probabilities (percentages) | |
|---|---|---|---|---|---|
| | | | | $p$ | $q$ |
| 1 | 9·09 | 86·36 | 4·55 | 95·45 | 9·52 |
| 2 | 32·58 | 64·25 | 3·17 | 96·83 | 33·65 |
| 3 | 35·08 | 63·74 | 1·17 | 98·82 | 35·50 |
| 4 | 26·55 | 73·23 | 0·21 | 99·78 | 26·61 |
| 5 | 36·51 | 63·49 | 0·00 | 100·00 | 36·51 |
| 6 | 22·97 | 70·27 | 6·76 | 93·24 | 24·64 |
| 7 | 25·00 | 75·00 | 0·00 | 100·00 | 25·00 |
| 8 | 19·81 | 80·18 | 0·00 | 99·99 | 19·81 |
| 9 | 18·08 | 80·14 | 1·77 | 98·22 | 18·41 |
| 10 | 18·77 | 80·06 | 1·17 | 98·83 | 18·99 |

TABLE 2

| Specimen no. | O/P | O/D | P/P | P/D | D/D |
|---|---|---|---|---|---|
| 2 | 13·02 | 47·95 | 0·68 | 10·95 | 27·39 |
| 4 | 26·45 | 48·06 | 0·32 | 8·39 | 16·77 |
| 9 | 21·92 | 56·68 | 0·00 | 6·42 | 14·97 |
| 10 | 20·35 | 56·64 | 0·44 | 3·54 | 19·03 |

Denoting by $n_1$, $n_2$, $n_3$, $n_4$, $n_5$, the observed percentages in the five classes O/P, O/D, P/P, P/D, D/D, the maximum likelihood estimators of $p_1$, $p_2$, $q_1$, $q_2$, are (appendix) $\hat{p}_1 = n_1+n_3+n_4-\Delta$ ; $\hat{p}_2 = n_2+n_5+\Delta$ ; $\hat{q}_1 = n_3+\Delta$ ; $\hat{q}_2 = n_4+n_5-\Delta$ where $\Delta = n_4p_2q_1/(p_2q_1+p_1q_2)$, so that $\Delta$ is a root of a certain cubic. A good approximation to $\Delta$ is

$$\frac{n_3n_4(n_2+n_5)}{n_3(n_2+n_5)+(n_1+n_3+n_4)(n_4+n_5)-n_4(n_2+n_3+n_5)}$$

Table 3 gives the estimates for the four specimens, together with the observed percentages and expected percentages calculated with these estimates.

The fit in each case is good. The smallness of the class P/P makes a $\chi^2$ test of fit rather unreliable on a total of about 200 (which is roughly the size of the counts made by Callan and Montalenti). However, the $\chi^2$ values calculated on a total of 200 are

| Specimen | $\chi^2_{[1]}$ | Probability |
|---|---|---|
| 2 | 0·1118 | $> 0·7$ |
| 4 | 0·3554 | $\doteq 0·6$ |
| 9 | 0·0502 | $> 0·8$ |
| 10 | 3·5834 | $\doteq 0·17$ |

The present hypothesis is therefore not significantly contradicted by the data, and as far as the *Culex* data can take us, it is admissible as an alternative explanation of the results, though at present more than this could not legitimately be claimed for it.

TABLE 3

| Spec. no. | | O/P | O/D | P/P | P/D | D/D | Estimated probabilities | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $p_1$ | $p_2$ | $q_1$ | $q_2$ | $q$ |
| 2 | Obs. | 13·02 | 47·95 | 0·68 | 10·95 | 27·39 | 21·23 | 78·87 | 4·10 | 34·92 | 39·02 |
| | Exp. | 12·94 | 48·09 | 0·87 | 10·64 | 27·54 | ... | ... | ... | ... | ... |
| 4 | Obs. | 26·45 | 48·06 | 0·32 | 8·39 | 16·77 | 34·67 | 63·33 | 0·81 | 24·67 | 25·48 |
| | Exp. | 25·84 | 48·68 | 0·28 | 9·08 | 16·12 | ... | ... | ... | ... | ... |
| 9 | Obs. | 21·92 | 56·68 | 0·00 | 6·42 | 14·97 | 28·34 | 71·66 | 0·00 | 21·39 | 21·39 |
| | Exp. | 22·28 | 56·32 | 0·00 | 6·19 | 15·33 | ... | ... | ... | ... | ... |
| 10 | Obs. | 20·35 | 56·64 | 0·44 | 3·54 | 19·03 | 23·95 | 76·05 | 0·82 | 22·19 | 23·01 |
| | Exp. | 18·44 | 58·55 | 0·20 | 5·94 | 16·88 | ... | ... | ... | ... | ... |

## 4. DISAPPEARANCE OF APPARENT INTERFERENCE WITH INCREASING ARM LENGTH

It has been shown that on the theory of a primary chiasma apparent interference is to be expected between arms, even if the centromere in fact operates as an insulator. The same theory has the merit of reconciling with one another the observations made by Callan and Montalenti on *Culex pipiens* and *Theobaldia longiareolata*, without assuming any essential difference between these two species, other than effective " length " in respect to chiasma frequencies. This is because, on quite general grounds, we may expect that the effect will be noticeable only in the case of very " short " chromosome arms as in *Culex*. Even if the phenomenon of the primary chiasma occurs, the spurious trans-centromeric interference will rapidly attenuate as arm length is increased. On the present theory, therefore, the difference between *Culex* and *Theobaldia* consists only in the difference in arm length as defined in terms of mean chiasma frequency.

Let $p$ be the probability of a primary chiasma being set up, so that $p$ is the frequency of chromosome pairs containing at least one chiasma ; *i.e.* the frequency of bivalent pairs.

Let now $q$ be the probability (conditional on the presence of the primary chiasma) of there being at least one chiasma in the arm not containing the primary. Then the classes : (*a*) univalent pairs, (*b*) bivalent pairs, (*c*) bivalents with chiasmata on both arms have the relative frequencies : $1-p$, $p$, $pq$.

We may expect that when the arms are " long," $p$ approximates to unity, and $q$ has a much larger value than in *Culex pipiens*. $q$ probably becomes almost as great as $p$. Hence in long-armed species the negative

correlation between chiasma numbers on the two arms tends to vanish. If it is desired to have a formal measure of the apparent interference, a suitable index can be defined in terms of a quantity analogous to the coefficient of genetical coincidence. We may take as index

$$I = 1 - C$$

where $$C = \frac{\text{frequency of bivalents with chiasmata on both arms}}{(\text{frequency of bivalents})^2}$$

$$= pq/(p)^2 = q/p.$$

Thus $$I = 1 - q/p = \frac{p-q}{p}.$$

On long arms we expect $q \to p \to 1$, $C \to 1$, and $I \to 0$.

It is altogether likely that the phenomenon, if it does exist, will rarely be noticed. Even if it should be universal, it will be very difficult to observe except in extraordinary species such as *Culex pipiens*. In normal species it is impossible to determine whether a primary chiasma is involved or not unless the data are very extensive. This can be well illustrated by analysing the data on *Theobaldia* in the light of the rival hypotheses.

## 5. ANALYSIS OF DATA ON *THEOBALDIA LONGIAREOLATA* MACQ.

In *Theobaldia longiareolata* Macq., Callan and Montalenti found three pairs of chromosomes, in many respects similar to those in *Culex pipiens*. Two pairs (M) are of approximately equal length and with approximately median centromeres. The third pair (*m*) is shorter in physical length, and has a much lower mean frequency of chiasma formation, in this resembling the chromosomes of *Culex*. The mean chiasma frequency per arm in the M bivalents was 1·456. The first two columns of table 4 show the types of M bivalents observed, and the frequencies of these types, and are taken from table 10 of Callan and Montalenti's paper.

Let A stand for the hypothesis of a primary chiasma. Let B denote the assumption that the first chiasmata to be formed on the two arms are on the same footing. In each case the centromere is supposed to act as an insulator.

The expected values for the class frequencies as calculated by Callan and Montalenti on hypothesis B, are given in column 3 of table 4. On this hypothesis the argument is as follows :—The arms are identical and independent. Considering a single arm, the probabilities of its containing 0, 1, 2, 3 chiasmata are respectively $1-r$, $r_1$, $r_2$, $r_3$ where $r_1+r_2+r_3 = r$. The expected class frequencies and the estimators of $r_1$, $r_2$, $r_3$ and $r$ are given in appendix II. The values obtained are $r_1 = 52{\cdot}65$ per cent., $r_2 = 42{\cdot}35$ per cent., $r_3 = 2{\cdot}94$ per cent., $r = 97{\cdot}94$ per cent. These values reproduce the expectations calculated by Callan and Montalenti.

Applying hypothesis A the argument is :—The relative frequencies of univalent pairs and bivalent pairs are $1-p$ and $p$, where $p$ is the probability of formation of the primary chiasma. $\frac{1}{2}p$ is the probability of the primary chiasma appearing on a particular arm. Let $\frac{1}{2}pp_1$, $\frac{1}{2}pp_2$, $\frac{1}{2}pp_3$, where $p_1+p_2+p_3 = 1$, be the respective probabilities of the primary chiasma appearing on a particular arm alone, or with one other chiasma, or with two other chiasmata.

TABLE 4

| Class | Observed | Expected on hypothesis B | Expected on hypothesis A |
|---|---|---|---|
| O/O | 0 | 0·07 | 0·00 |
| O/X | 5 | 3·69 | 4·14 |
| O/XX | 2 | 2·96 | 2·45 |
| O/XXX | 0 | 0·20 | 0·41 |
| X/X | 51 | 47·12 | 46·39 |
| X/XX | 69 | 75·80 | 77·45 |
| X/XXX | 3 | 5·25 | 4·61 |
| XX/XX | 33 | 30·48 | 29·57 |
| XX/XXX | 7 | 4·23 | 4·98 |
| XXX/XXX | 0 | 0·15 | 0·00 |

$\chi^2_{[6]} = 5{\cdot}1112$ $\chi^2_{[3]} = 3{\cdot}8322$

Let $1-q$, and $q$ be the probabilities (conditional on the existence of a primary in one arm) of the non-appearance or appearance of at least one chiasma in the other arm. Let $q_1$, $q_2$, $q_3$, where $q_1+q_2+q_3 = q$, be the conditional probabilities of there being exactly 1, 2 or 3 secondaries on this other arm. Then the expectations are as given in appendix II. Applying the estimation formulæ given there to the data, we get as estimators of the parameters

$$\hat{p}_1 = 0{\cdot}329{,}412+\Delta \qquad \hat{q}_1 = 0{\cdot}723{,}529-\Delta$$
$$\hat{p}_2 = 0{\cdot}611{,}765-\Delta \qquad \hat{q}_2 = 0{\cdot}235{,}294+\Delta$$
$$\hat{p}_3 = 0{\cdot}058{,}824 \qquad \hat{q}_3 = 0{\cdot}0$$
$$\hat{p} = 1{\cdot}000{,}001 \qquad \hat{q} = 0{\cdot}958{,}823$$

where $\Delta$ is the relevant root of

$$2\Delta^3-1{\cdot}176{,}470\,\Delta^2+0{\cdot}290{,}935\,\Delta-0{\cdot}031{,}460 = 0.$$

By trial it is found that, correct to four figures, $\Delta = 0{\cdot}2622$ Consequently

$$\hat{p}_1 = 59{\cdot}16 \text{ per cent.} \qquad \hat{q}_1 = 46{\cdot}13 \text{ per cent.}$$
$$\hat{p}_2 = 34{\cdot}96 \text{ ,,} \qquad \hat{q}_2 = 49{\cdot}75 \text{ ,,}$$
$$\hat{p}_3 = 5{\cdot}88 \text{ ,,} \qquad \hat{q}_3 = 0{\cdot}00 \text{ ,,}$$
$$\hat{p} = 100{\cdot}00 \text{ ,,} \qquad \hat{q} = 95{\cdot}88 \text{ ,,}$$

It may be noted, incidentally, that these values satisfy the relations $\hat{r}_i = \frac{1}{2}(\hat{p}_i+\hat{q}_i)$, $\hat{r} = \frac{1}{2}(\hat{p}+\hat{q})$. The corresponding expectations are given in column 4 of table 4. They are seen to give what is, on the

whole, about as good a fit to the observations as do those calculated on hypothesis B. The small classes are reproduced with almost consistently greater accuracy, but the large class numbers are more divergent on A than on B, though the discrepancies are in the same directions as those arising on hypothesis B. In so far as a $\chi^2$ test is applicable or informative we have :—

$$\text{On hypothesis A } \chi^2_{[3]} = 3{\cdot}8322,\ Pr = 0{\cdot}28,$$
$$\text{On hypothesis B } \chi^2_{[6]} = 5{\cdot}1112,\ Pr > 0{\cdot}50.$$

Clearly no decision between hypotheses could legitimately be made, since neither is significantly better than the other.

The formal index of interference between arms (as defined in section 4) is zero on hypothesis B, and 0·0412 on hypothesis A.

In passing it may be noted that on the basis of the $r$ values (or of the mean $p$ and $q$ values) the intensity of chiasma interference, as defined by Haldane (1931), has the value

$$1 - \frac{\text{variance of } m}{\text{mean of } m} = 0{\cdot}761$$

(where $m$ is the number of chiasmata per arm), which is of quite the usual order of magnitude. Hence the M chromosomes of *Theobaldia* appear to be perfectly typical ones in respect of chiasma frequency and intensity of chiasma interference. The present discussion shows therefore that the quasi-interference due to a primary chiasma is likely to be quite invisible in typical chromosomes, and could only make itself apparent in abnormally " short " chromosomes like those of *Culex*.

## Appendix I

## ESTIMATION OF PARAMETERS FOR *CULEX*

We have

| | O/P | O/D | P/P | P/D | D/D |
|---|---|---|---|---|---|
| Observed | $Nn_1$ | $Nn_2$ | $Nn_3$ | $Nn_4$ | $Nn_5$ |
| Expected | $p_1(1-q_1-q_2)$ | $p_2(1-q_1-q_2)$ | $p_1q_1$ | $p_2q_1+p_1q_2$ | $p_2q_2$ |

(multiplied by $Np$).

Since $p_1+p_2 = 1$, the function to be maximised is

$$Nn_1 \log p_1 (1-q_1-q_2)+Nn_2 \log p_2(1-q_1-q_2)+Nn_3 \log p_1q_1 +Nn_4 \log (p_2q_1+p_1q_2)+Nn_5 \log p_2q_2-N\lambda(p_1+p_2).$$

Therefore the estimators satisfy

$$n_1+n_3+\frac{n_4p_1q_2}{p_2q_1+p_1q_2} = \lambda p_1\ ;\ -\frac{(n_1+n_2)q_1}{1-q_1-q_2}+n_3+\frac{n_4p_2q_1}{p_1q_1+p_1q_2} = 0$$

$$n_2+n_5+\frac{n_4p_2q_1}{p_2q_1+p_1q_2} = \lambda p_2\ ;\ -\frac{(n_1+n_2)q_1}{1-q_1-q_2}+n_5+\frac{n_4p_1q_2}{p_1q_2+p_2q_1} = 0$$

which give $\lambda = n_1+n_2+n_3+n_4+n_5 = 1$

and $\hat{q} = \hat{q}_1+\hat{q}_2 = n_3+n_4+n_5.$

Putting $$\Delta = n_4 p_2 q_1/(p_2 q_1 + p_1 q_2) \quad . \quad . \quad . \quad . \quad (1)$$

we get $p_1 = n_1 + n_3 + n_4 - \Delta$, $p_2 = n_2 + n_5 + \Delta$,

$q_1 = n_3 + \Delta$ , $q_2 = n_4 + n_5 - \Delta$.

Substituting in (1), $\Delta$ is found to satisfy a cubic, of which the relevant root is approximately equal to

$$\Delta = \frac{n_3 n_4 (n_2 + n_5)}{n_3(n_2+n_5) + (n_1+n_3+n_4)(n_4+n_5) - n_4(n_2+n_3+n_5)}.$$

This value can be rapidly improved by iteration.

## Appendix II

## ESTIMATION OF PARAMETERS FOR *THEOBALDIA*

### *Hypothesis A: primary chiasma*

The normalised class frequencies are :—

| Class | Observed | Expected |
|---|---|---|
| O/O | $n_1$ | $1-p$ |
| O/X | $n_2$ | $pp_1(1-q)$ |
| O/XX | $n_3$ | $pp_2(1-q)$ |
| O/XXX | $n_4$ | $pp_3(1-q)$ |
| X/X | $n_5$ | $pp_1q_1$ |
| X/XX | $n_6$ | $p(p_1q_2+p_2q_1)$ |
| X/XXX | $n_7$ | $p(p_1q_3+p_3q_1)$ |
| XX/XX | $n_8$ | $pp_2q_3$ |
| XX/XXX | $n_9$ | $p(p_2q_3+p_3q_2)$ |
| XXX/XXX | $n_{10}$ | $pp_3q_3$ |

where $p_1+p_2+p_3 = 1$ and $q = q_1+q_2+q_3$. The function to be maximised is

$$n_1 \log (1-p) + n_2 \log pp_1(1-q) + \ldots . - \lambda(p_1+p_2+p_3).$$

The estimators satisfy

$$\frac{n_1}{1-p} = \frac{\sum_2^{10} n_i}{p}$$

$$\begin{cases} \lambda p_1 = n_2+n_5 + \Delta_2+\Delta_3 \\ \lambda p_2 = n_3+n_6+n_8 + \Delta_1 - \Delta_3 \\ \lambda p_3 = n_4+n_7+n_9+n_{10} - \Delta_1 - \Delta_2 \end{cases}$$

$$\begin{cases} (n_2+n_3+n_4)\frac{q_1}{1-q} = n_5+n_6+n_7 - \Delta_2 - \Delta_3 \\ (n_2+n_3+n_4)\frac{q_2}{1-q} = n_8+n_9 - \Delta_1 + \Delta_3 \\ (n_2+n_3+n_4)\frac{q_3}{1-q} = n_{10} + \Delta_1 + \Delta_3 \end{cases}$$

where $\Delta_1 = \frac{n_9 p_2 q_3}{p_2 q_3 + p_3 q_2}$, $\Delta_2 = \frac{n_7 p_1 q_3}{p_1 q_3 + p_3 q_1}$, $\Delta_3 = \frac{n_6 p_1 q_2}{p_1 q_2 + p_2 q_1}$

Therefore $$\lambda = 1-n_1, \quad \hat{q} = \sum_5^{10} n_i \div (1-n_1)$$

$$\begin{cases} (1-n_1)\hat{p}_1 = n_2+n_5 & +\Delta_2+\Delta_3 \\ (1-n_1)\hat{p}_2 = n_3+n_6+n_8 & +\Delta_1 \quad -\Delta_3 \\ (1-n_1)\hat{p}_3 = n_4+n_7+n_9+n_{10} & -\Delta_1-\Delta_2 \end{cases}$$

$$\begin{cases} (1-n_1)\hat{q}_1 = n_5+n_6+n_7 & -\Delta_2-\Delta_3 \\ (1-n_1)\hat{q}_2 = n_8+n_9 & -\Delta_1 \quad +\Delta_3 \\ (1-n_1)\hat{q}_3 = n_{10} & +\Delta_1 \quad +\Delta_3 \end{cases}$$

If $n_1$ and $n_{10}$ are zero, then the solution is

$$\begin{cases} \hat{p}_1 = n_2+n_5 & +\Delta \\ \hat{p}_2 = n_3+n_6+n_8 & -\Delta \\ \hat{p}_3 = n_4+n_7+n_9 \end{cases}$$

$$\begin{cases} \hat{q}_1 = n_5+n_6+n_7 & -\Delta \\ \hat{q}_2 = n_8+n_9 & +\Delta \\ \hat{q}_3 = 0 \end{cases}$$

where $\Delta_1 = \Delta_2 = 0$, $\Delta_3 = \Delta$, and $\Delta$ satisfies the cubic

$$2\Delta^3+\Delta^2(n_2-n_3-3n_6-n_7+n_9)$$
$$+\Delta[(n_2+n_5)(n_8+n_9)+(n_3+n_6+n_8)(n_5+n_6+n_7)-n_6(n_2+n_5+n_8+n_9)]$$
$$-n_6(n_2+n_5)(n_8+n_9) = 0$$

which is easily solved by trial.

*Hypothesis B : chiasmata on equal footing*

The normalised class frequencies are :—

| | | |
|---|---|---|
| O/O | $n_1$ | $(1-r)^2$ |
| O/X | $n_2$ | $2r_1(1-r)$ |
| O/XX | $n_3$ | $2r_2(1-r)$ |
| O/XXX | $n_4$ | $2r_3(1-r)$ |
| X/X | $n_5$ | $r_1^2$ |
| X/XX | $n_6$ | $2r_1r_2$ |
| X/XXX | $n_7$ | $2r_1r_3$ |
| XX/XX | $n_8$ | $r_2^2$ |
| XX/XXX | $n_9$ | $2r_2r_3$ |
| XXX/XXX | $n_{10}$ | $r_3^2$ |

where $$r_1+r_2+r_3 = r.$$

The estimators are :—

$$\hat{r}_1 = \tfrac{1}{2}(n_2+2n_5+n_6+n_7)$$
$$\hat{r}_2 = \tfrac{1}{2}(n_3+n_6+2n_8+n_9)$$
$$\hat{r}_3 = \tfrac{1}{2}(n_4+n_7+n_9+2n_{10})$$
$$\hat{r} = 1-n_1-\tfrac{1}{2}(n_2+n_3+n_4).$$

These are in a simple relation to the estimators under hypothesis A. Since

$$\hat{r}_i = (1-n_1)\tfrac{1}{2}(\hat{p}_i+\hat{q}_i), \ (i = 1, 2, 3).$$

## 6. SUMMARY

It is shown that the observations of Callan and Montalenti are not only consistent with the hypotheses advanced by these writers, namely :—chiasma interference operates across the centromere in *Culex pipiens*, but is confined to the interior of each arm in the chromosomes of *Theobaldia longiareolata* : but may also be explained with equal legitimacy on the hypothesis that a primary chiasma is formed with a high probability, and that subsequent chiasmata are conditional on its existence and are not on the same footing as the primary.

It is shown that when the centromere acts as an interference insulator, the occurrence of the primary chiasma has the effect of an apparent chiasma interference between arms. The effect is marked in " short " chromosomes, but is very much less in arms of normal length. In the latter case it is almost imperceptible.

On the present data this explanation can only be put forward as a possible alternative to the explanation in terms of real interference. It has, however, the slight advantage of representing an economy of hypotheses. It requires that the chromosomes of *Culex pipiens* be exceptional only in respect of the low mean frequency of chiasmata per arm of bivalent, and not exceptional *both* in this respect, and in that of having a centromere transparent to chiasma interference.

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# CHROMOSOME NUMBERS OF SOUTH AFRICAN GRASSES

By A. A. MOFFETT
*Wattle Research Institute, University of Natal*
and
R. HURCOMBE
*John Innes Horticultural Institution, Bayfordbury*

THE following list of chromosome numbers is a combination of the studies of Dr Moffett on grasses of Rhodesia and Miss Hurcombe on grasses of the provinces of the Union of South Africa. Dr Moffett's species were identified by Miss K. Sturgeon of the Department of Agriculture, Salisbury, Southern Rhodesia ; Miss Hurcombe's species were grown from seed supplied by Miss Wilman through the Director, Royal Botanic Gardens, Kew, or roots were sent directly from South Africa, and specimens of these have been preserved in the Kew Herbarium (Editor).

Notes after the numbers refer to comparison with previous counts given in the *Chromosome Atlas* where the full references to the original papers will be found.

TRIBE II.—*ANDROPOGONEÆ*

| | | | |
|---|---|---|---|
| $x = 10$ | ROTTBOELLIA | | |
| | *exaltata* L. f. . . . | 20 | S. Rhodesia |
| | SORGHUM | | |
| | *sudanense* (Piper) Stapf . | 20 | ,, |
| | *friesii* (Pilger) C. E. Hubbard (*micratherum* Stapf) | 40 | ,, |
| | SCHIZACHYRIUM | | |
| | *glabrescens* (Rendle) Stapf . | 20 | ,, |
| | *jeffreysii* (Hack.) Stapf . . | 40 | ,, |
| | DIECTOMIS | | |
| | *fastigiata* (Swartz) Kunth . | 20 | ,, |
| | ANDROPOGON | | |
| | *eucomus* Nees . . . | 20 | ,, |
| | *schirensis* Hochst. ex A. Rich . | 40 | ,, |
| | *amplectens* Nees . . . | 40 | ,, |
| | *schinzii* Hack . . . | 40 | ,, |
| | *gayanus* Kunth *var. squamulatus* (Hochst.) Stapf | 40 | ,, |
| | HYPARRHENIA | | |
| | *dissoluta* (Nees ex Steud.) C. E. Hubbard (*ruprechtii* Fourn.) | 40 | ,, |
| | *newtonii* (Hack.) Stapf . . | 40 | ,, |
| | MONOCYMBIUM | | |
| | *ceresiiforme* (Nees) Stapf . | 20 | ,, |

| | | | |
|---|---|---|---|
| $x = 11$ | HETEROPOGON | | |
| | *melanocarpus* (Ell.) Benth. | 22 | *S. Rhodesia* |
| | *contortus* (L.) Beauv. ex R. & S. | 44 (not 20) | ,, |
| | **Tribe III.—*PANICEÆ*** | | |
| $x = 7, 9$ | UROCHLOA | | |
| | *pullulans* Stapf | 28 | ,, |
| | *mosambicensis* (Hack.) Dandy (= U. *pullulans var. mosambicensis* Stapf) | 42 | ,, |
| | *bolbodes* (Schweinf.) Stapf | 36 | ,, |
| | *panicoides* Beauv. (*helopus* Stapf) | 36 | ,, |
| $x = 8, 10, 11$ | PANICUM | | |
| | *maximum* Jacq. | 32 (not 36) | ,, |
| | Dwarf type and green mutant | 32 | ,, |
| | *vars.* Red Buffel, Coarse Guinea, Fine Guinea | 32 | ,, |
| | two *vars.* Puerto Rico | 32 | Inst. Trop. Ag. |
| | "*makarikari*" | 44 | S. Rhodesia |
| $x = 9$ | DIGITARIA | | |
| | *milanjiana* (Rendle) Stapf | 18 | ,, |
| | *brazzæ* (Franch.) Stapf | 18 | ,, |
| | *gazensis* Rendle | 18 | ,, |
| | *eriantha* Steud. | 18 | ,, |
| | *pentzii* Stent | 54 | ,, |
| | *swazilandensis* Stent | 18 | Frankenwald Turf Nursery, Transvaal |
| $x = 9, 17$ | CENCHRUS | | |
| | *ciliaris* L. | 36 (not 34) | S. Rhodesia |
| $x = 9$ | CHLORIDION | | |
| | *cameronii* Stapf | 54 | ,, |
| | ALLOTEROPSIS | | |
| | *semialata* (R. Br.) Hitch. | 54 | ,, |
| $x = 9, 21$ | BRACHIARIA | | |
| | *viridula* Stapf | 36 | ,, |
| | *nigropedata* (Munro) Stapf | 36 | ,, |
| | *serrata* (Thunb.) Stapf | 36 | ,, |
| | *brizantha* (Hochst. ex A. Rich.) Stapf | 54 | ,, |
| | *dictyoneura* (Fig. et De Not.) Stapf | 42 | ,, |
| $x = 9$ | SACCIOLEPIS | | |
| | *glaucescens* Stapf | 36 | ,, |
| $x = 9$ | SETARIA | | |
| | *longiseta* Beauv. | 18 | ,, |
| | *phragmitoides* Stapf | 36 | ,, |
| | *pallide-fusca* (Schumach.) Stapf et Hubbard | 36 | ,, |
| | *chevalieri* Stapf | 54 | ,, |
| | *sphacelata* (Schumach.) Stapf et Hubbard | 36, 54 | Rietondale, Transvaal |
| | *sphacelata var.* Kasingulu | 36 | ... |
| | *sphacelata var.* Gomoti River | 36 | ... |
| | *splendida* Stapf * | 63 (7*x*) | Rietondale, Transvaal |

* *Setaria splendida*—a tall vigorous grass which does not set viable seed—proved to be heptaloid.

| | | | |
|---|---|---|---|
| | RHYNCHELYTRUM | | |
| | *repens* (Willd.) C. E. Hubbard (*roseum* (Nees) Stapf et Hubbard) | 36 | S. Rhodesia |
| | *nyassanum* (Mez) Stapf et Hubbard | 36 | ,, |
| | *setifolium* (Stapf) Chiov. | 36 | ,, |
| | *minutiflorum* (Rendle) Stapf et Hubbard | 36 | ,, |
| | *minutiflorum var.* melinoides (Stent) | 36 | ,, |
| | MELINIS | | |
| | *macrochæta* Stapf et Hubbard | 36 | ,, |
| | ACROCERAS | | |
| | *macrum* Stapf | 36 ($n = 18$) | Rietondale, Transvaal |
| $x = 10$ | PASPALUM | | |
| | *commersonii* Lam. (*scorbiculatum* L. *var. commersonii* Stapf) | 40 | S. Rhodesia |

TRIBE IIIA.—*ARUNDINELLEÆ*

| | | | |
|---|---|---|---|
| $x = 10$ | LOUDETIA | | |
| | *simplex* (Nees) C. E. Hubbard (*Trichopteryx simplex* Hack.) | 60 | ,, |
| | TRISTACHYA | | |
| | *welwitschii* Rendle *var. superbiens* (Pilger) C. E. Hubbard | 40 | ,, |

TRIBE IV.—*ERAGROSTEÆ*

| | | | |
|---|---|---|---|
| $x = 10, 21$ | POGONARTHRIA | | |
| | *squarrosa* (Licht.) Pilger and *var.* | 40 | ,, |
| | *squarrosa* | 42 | Kimberley, Cape |
| $x = 10$ | TETRACHNE | | |
| | *dregei* Nees * | 20 | Middelburg, Cape |
| | ERAGROSTIS | | |
| | *aspera* (Jacq.) Nees | 20 | S. Rhodesia |
| | *aspera* | 20 † | Kimberley, Cape |
| | *biflora* Hack. I | 20 | Mafeking, Cape |
| | *biflora* Hack. II | 20 | Kimberley, Cape |
| | *chapelieri* Nees | 20 | ... |
| | *cilianensis* (All.) Lutati | 20 † | Irene, Transvaal |
| | *namaquensis* Nees | 20 | ... |
| | *pallens* Hack. | 20 | Andalusia, N. Cape |
| | *patens* Oliv. | 20 | ... |
| | *truncata* Hack. | 20 | Fauresmith, O.F.S. |

* *Tetrachne* is a monotypic genus which has not previously been studied. Stapf (*Flora Capensis*, 7, 318, 1898) places this genus in the tribe Festuceæ. The somatic chromosomes of true members of the Festuceæ, e.g. *Festuca*, *Poa* and *Dactylis* are twice as long as those of *T. dregei*. Also, the majority of the Festuceæ have a basic number of 7 whereas the count for *T. dregei* indicates a basic number of 10. Mr C. E. Hubbard has found that this grass differs from the genera of the tribe Festuceæ and from allied tribes of the festucoid group in certain morphological and anatomical features. The morphological, anatomical and cytological evidence both indicate that *Tetrachne* should be removed from the Festuceæ. For the present the genus might be conveniently included in the Eragrosteæ.

† Confirming Avdulov 1931.

| | | | |
|---|---|---|---|
| $x = 10$ | ERAGROSTIS—*continued* | | |
| | *wilmaniæ* Hubb. et Schw. | 20 | Hay Division, Griqualand West |
| | *atherstonei* Stapf I | 40 | Mafeking, Cape |
| | *atherstonei* Stapf II | 40 | ,, ,, |
| | *ciliaris* (L.) R. Br. | 40 | ... |
| | *denudata* Hack. | 40 | ... |
| | *echinochloidea* Stapf | 40 | Barkly West, Cape |
| | *margaritacea* (?) | 40 | ... |
| | *obtusa* Munro | 40 | Kimberley, Cape |
| | *sclerantha* Nees (small type) | 40 | S. Rhodesia |
| | *sclerantha* Nees (large type) | 40 | ,, |
| | *superba* Peyr. | 40 | Kimberley, Cape |
| | *tef* (Zucc.) Trotter (*E. abyssinica* Link) | 40 | S. Rhodesia |
| | *viscosa* (Retz.) Trin. | 40 | ,, |
| | *barrelieri* Daveau | 60 | Kimberley, Cape |
| | *capensis* Trin. (*brizoides* Nees) | 60 | S. Rhodesia |
| | *habrantha* Rendle | 60, 90 (6$x$ and 9$x$) | ,, |
| | TRIBE V.—*SPOROBOLEÆ* | | |
| $x = 9, 10$ | SPOROBOLUS | | |
| | *fimbriatus* Nees | 18 | Kimberley, Cape |
| | *panicoides* A. Rich. | 24 | S. Rhodesia |
| | *pyramidalis* Beauv. | 24 | ,, |
| | *pyramidalis* | 30 (3$x$) | Irene, Transvaal |
| | *capensis* (Willd.) Kunth | 36 | S. Rhodesia |
| | TRIBE VI.—*ZOISIEÆ* | | |
| $x = 10$ | PEROTIS | | |
| | *patens* Gand. | 40 | S. Rhodesia |
| | TRIBE VII.—*CHLORIDEÆ* | | |
| $x = 9, 10$ | CRASPEDORHACHIS | | |
| | *rhodesiana* Rendle | 27 (3$x$) | S. Rhodesia |
| | ELEUSINE | | |
| | *indica* (L.) Gaertn.* | 36 (not 18) | ,, |
| | DACTYLOCTENIUM | | |
| | *ægytium* (L.) Beauv. | 36 | ,, |
| | CYNODON | | |
| | *dactylon* (L.) Pers. | 40 | ,, |
| | *plectostachyum* (K. Schum.) Pilger | 18, 54 | ,, |
| $x = 9, 10$ | CHLORIS | | |
| | *gayana Kunth* | 20, 40 | ,, |
| | ,, ,, *var.* Trans Nzoia | 20 | ,, |
| | ,, ,, *var.* Giant Rhodes Grass | 40 | ,, |
| | ,, ,, *var.* Kafue Strain | 40 | ,, |
| | ,, ,, *var.* Hunyani Grass | 40 | ,, |

* *Eleusine indica* $2n = 36$ and not $2n = 18$. This may be tetraploid strain. It is generally considered that this species becomes larger and more aggressive as one proceeds north from S. Africa. It is certainly much more formidable in Rhodesia than in Natal, but this may be due to climate and soil as much as to any inherent differences.

| | | | |
|---|---|---|---|
| $x = 9,10$ | CHLORIS—*continued* | | |
| | *virgata* Swartz * . . . | 20 (not 14) | Mafeking, Cape |
| | *pycnothrix* Trin. . . . | 40 | Irene, Transvaal |
| | MICROCHLOA | | |
| | *Kunthii* Desv. . . . | 40 | S. Rhodesia |
| | TRICHONEURA | | |
| | *grandiglumis* (Rendle) Ekman | 20 | ,, |
| | TRIBE IX.—*ARISTIDEÆ* | | |
| $x = 11$ | ARISTIDA | | |
| | *meridionalis* Henrard . . | 22 | S. Rhodesia |
| | *scabrivalvis* Hack. . . . | 22 | ,, |
| | *macilenta* Henrard (*contractinodis* Stent et Rattray) | 22 | ,, |
| | *congesta* Roem. et Schult. . | 22 | ,, |
| | *scabrivalvis* (?) . . . | 22 | ,, |
| | *leucophæa* Henrard . . | 44 | ,, |

* Nielsen and Humphrey (1937) counted 14 chromosomes in *Chloris virgata*. The present revised count of $2n = 20$ agrees with previous counts of other species of *Chloris*.

*N.B.*—The manuscripts have been arranged and edited by Mr C. E. Hubbard of the Royal Botanic Gardens, Kew, and Dr C. D. Darlington.

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# REVIEW

**BIOMETRICAL GENETICS. By K. Mather. London : Methuen, 1949. Pp. ix+162. 18s**

Professor Mather commences by reviewing the historical development leading up to the fusion of Biometry and Mendelism in biometrical genetics. Genetics has concerned itself mainly with the study of discontinuous variation, and in the wide variety of instances where the phenotypic classes can be shown to have an obvious correspondence with the inheritance of relatively unchanging and independently assorting units, the success of the Mendelian approach and analysis has been enormous. Prior to the rediscovery of Mendel's work in 1900 genetical investigations had been started by Galton and continued by Pearson. But the concentration on human material with its small families of doubtful ancestry, as well as the choice of quantitative characters, prevented discovery of the method of transmission. Galton and Pearson had shown that some continuous variation was partly inherited and Darwin had stressed the importance of small cumulative changes for the theory of evolution. The problem of continuous variation could not be ignored. With the new recognition of the importance of Mendel's work there was some initial rivalry between the Biometricians and the Mendelians which delayed synthesis of the two approaches. The multiple factor hypothesis, already considered by Mendel, was advocated as early as 1907 by G. U. Yule, and in 1910 by E. M. East ; while Nilsson-Ehle's work (1909-11) on the trifactorial inheritance of grain colour in wheat was a case of special influence in the matter. In 1909 Johannsen showed that both heritable and non-heritable factors contributed to the variation of seed weight in beans, and that the effects were of the same order of magnitude. The stage was then set for the emergence of a polygenic theory of inheritance embodying both the Mendelian basic assumptions and the biometrical or statistical method of analysis. In 1918 an important step forward was made when Professor R. A. Fisher showed how the results of the biometricians, particularly the correlations observed in human families, followed from the new approach ; Fisher also produced evidence of dominance relationships. At this point Mather goes on to discuss in some detail the evidence for polygenic segregation and linkage, and the relation between polygenes and the " major " genes of Mendelian genetics.

In the second chapter, entitled " Characters," the relations between genotype and phenotype in polygenic systems are considered, and a method is described of finding a function of a number of metrical characters which shall be " best " in the sense of being as sensitive as possible to *genetical* variation. This is followed by a chapter devoted to the fundamentally important question of " Scaling." It is obvious that with a metrical character there must be a variety of scales of measurement to choose from. A satisfactory scale will be one for which both the non-heritable effects and the individual genic contributions are additive, or approximately so. Tests are given for the adequacy of a given scale, based on the homogeneity of different estimates of the residual error variance.

A certain amount of statistical knowledge is essential in the treatment of the subject, though it need not be extensive. Mather assumes some acquaintance with both genetics and statistics, but is careful to introduce the mathematics gradually. It is not until the fourth chapter that he embarks upon the fundamental method of analysis developed in 1932 by Fisher, Immer and Tedin. This is a procedure for partitioning the variation in filial and backcross generations into genetic and environmental portions by considering certain functions of means, variances, covariances, etc. This leads on to the question of linkage, which is dealt with in the succeeding chapter. Although linkage does not influence mean values it does affect the second degree statistics. Thus the test for linkage is essentially a test of homogeneity of the genetic components of variation among the available filial and backcross data.

Mather then proceeds to a discussion of the rather more sophisticated concept of " Effective Factors." The actual number of genes operating in a polygenic system is somewhat difficult to arrive at. Its estimate is complicated by the incomplete concentration of " similar " allelomorphs in the parents ; by the incomplete reinforcement of " unfixable " components ; by the inequality of the genetic increments ; and by the presence of linkage. In 1940 Panse devised a method of coping with the first three difficulties ; he has also used his analysis to tackle the vitally important problem of the speed of advance under selection. If linkage is very tight or very loose then evidence of its existence may be hard to come by. In ordinary Mendelian genetics the linkage of two genes, previously considered to be a single gene of pleiotropic effect, may be suggested by a single rare instance of recombination. But in biometrical genetics recombination can only be detected if its consequences admit of *statistical* recognition. The upshot is that the biometrical units in terms of which the data are described may easily be not actual genes but a certain number of *effective* factors.

In the final chapter Mather concludes with a discussion of experimental design, and of the general ideas involved in the subject.

Mather's book, which is the first of its kind, will be welcomed by all geneticists as showing just how the basic ideas of Mendelian genetics, so appropriate to discrete variation, can also be used to lay the foundations of a biometrical genetics of quantitative characters. The many worked examples and the references to the literature, including the pioneer work of Mather himself, will be found especially useful. The great value of the book lies in its making more widely known methods of dealing with continuous variation, which is of cardinal importance for a variety of subjects such as : the social consequences of the conditions of inheritance of human intelligence ; the theory of evolution ; and the practical problems of plant and animal breeding.

NORMAN T. J. BAILEY.

# GENETICAL SOCIETY OF GREAT BRITAIN

## TITLES AND SUMMARIES OF DEMONSTRATIONS given at the HUNDREDTH MEETING OF THE SOCIETY, held in CAMBRIDGE, on 29th and 30th JUNE and 1st JULY 1949.

### AUTO-SEXING BREEDS OF POULTRY

M. S. PEASE
*Small Animal Breeding Research Station, Cambridge*

The sex-linked Barred gene is present in several well-established varieties of poultry. In all these breeds it occurs in association with black down and plumage. The Barred gene produces a light spot on the back of the head of the chick, which shows up clearly on the otherwise black down. Being sex-linked, this gene is duplex in the male and simplex in the female chick. But generally speaking, it is not possible *in the presence of the Black gene* to distinguish between the homozygous and the heterozygous light head patch : dominance is perfect. But in the absence of the Black gene, as in Brown Leghorns for example, the dominance of the Barred gene becomes imperfect as far as concerns the light head patch. The enormously extended condition of the homozygous (male) light head patch compared with the heterozygous (female) light head is at once clear at hatching, when the dominant Black gene is removed.

Several new varieties on this principle, have been made at Cambridge, by transferring the Barred gene from a black to a non-black breed. These new breeds have been graded up and are now finding their way into commercial use. Examples of these are shown at the Animal Research Station, Huntingdon Road.

### *LYTHRUM SALICARIA* OPEN FERTILISATION PLOT

R. A. FISHER *et al.*
*Department of Genetics, Cambridge*

Framework of recessive pollen-parents, pink, long-styled, for fertilising short-styled seed-parents, usually purple, the genotype of which is thus tested. Usually a hundred or more seed-parents are tested each year as second backcrosses to the triple recessive. A few plants of known genotype are also kept as sources of first backcrosses.

### *OXALIS VALDIVIENSIS*

Portion of progeny bred for the evaluation of linkage between mid and short genes. The linkage is in coupling with expectation about 14 long : 1 mid : 15 short. All three style types should thus be available.

### *PISUM SATIVUM*

Progenies segregating for four of the plant characters and the two seed characters used by Mendel.

### GENETICS OF MICRO-ORGANISMS

There is a new bacteriological laboratory in the department, and Dr Cavalli will be pleased to show the work in progress to a few people at a time.

## GENETICS OF THE HOUSE MOUSE

Small parties may also be interested in the factors used in genetic work with mice.

## POLYPLOID MOUSE EGGS

R. A. BEATTY and M. FISCHBERG
*Animal Breeding and Genetics Research Organisation, Edinburgh*

Eggs from the morula to late blastula stage were examined and found to be diploid in most mouse stocks. In one particular stock, however, there was a spontaneous occurrence of polyploidy (mainly triploidy) in 6 per cent. of the eggs and 20 per cent. of the mice examined. Polyploidy could also be induced experimentally by warming eggs *in vivo* at certain critical temperatures under conditions designed to prevent cell division at specific times in development. Eggs treated at the time when they were expected to contain the maternal chromosomes in *diploid* number and a sperm developed in a high proportion of cases into triploids.

Treatment at the expected time of the first cleavage metaphase gave tetraploid eggs. These embryos, which have been examined up to the late blastula stage are the most advanced polyploid mammals known at present.

## CULTURAL METHODS FOR THE MYCETOZOON *DIDYMIUM NIGRIPES*

E. A. BEVAN
*Department of Genetics, University, Glasgow*

Demonstration of the various stages in the life-cycle: (1) myxoamoebae; (2) plasmodia; (3) fruiting bodies.

Growth of *Didymium* in two-member culture, with *Escherichia coli* as food.

Elimination by means of streptomycin of *E. coli* from the two-member culture in order to study the genetics of nutritional requirements in *Didymium*.

## EXPERIMENTAL STUDIES ON ISOLATED AMPHIBIAN OOCYTE NUCLEI

H. G. CALLAN
*Institute of Animal Genetics, Edinburgh*

The physical properties and chemical nature of the nuclear membrane have been examined by a variety of techniques. The membrane is a double structure: on the outside is a porous sheet, thickness 500 Angstrom, with pores 300 Angstrom wide, repetition distance 800 Angstrom, the pores being uniform and in hexagonal close packing; on the inside is a "structureless" protein membrane, thickness 100 Angstrom, with remarkable elastic, adhesive and hydrophobic qualities. The permeability properties of this membrane are under examination.

The colloidal properties and chemical nature of the nuclear sap has also been examined by a variety of techniques. The nuclear sap consists of structural and disperse phases of a protein or proteins rich in cyclic amino-acids; nucleic acids are absent, though ribose nucleic acid is present in high concentration in the nucleoli.

A start has been made on a study of the metabolic activity of isolated nuclei.

## THE DEVELOPMENT OF TIBIAL HEMIMELIA IN MICE

T. C. CARTER
*Institute of Animal Genetics, Edinburgh*

Tibial hemimelia in mice is due to a single mutant in Linkage Group III: recombination with macrocytic anæmia, $W^v$, is 16 per cent. and with piebald spotting,

*s*, is 50 per cent. The heterozygote may be normal or may show preaxial polyphalangy or polydactyly of one or both the hind feet; occasionally there is a reduction of the distal part of the tibia. The homozygote shows similar or more severe defects; tibia and pubis may be absent, the femur represented only by its epiphyses; the kidneys may be hydrotic.

Limb defects are clearly identifiable in homozygous embryos of 12½ days' gestation: there is excessive growth of the proximal preaxial part of the hind footplate. Abnormally narrow and pointed limb buds can be seen in some embryos of 11½ and 12 days, before the differentiation of the footplate, and may represent an earlier stage of the defect. The condition is thus due to abnormal mesenchymal condensation. A similitude of faulty chondrification is found in some later embryos but is due to abortive condensation of the tibia anlage; after the outer shell of condensed mesenchyme has formed perichondrium there is no inner core left, such as would normally form cartilage, so the tibia appears to be replaced by a ligament; this, however, represents cartilage-less perichondrium.

## ICHTHYOTIC MICE

T. C. CARTER and R. S. PHILLIPS
*Institute of Animal Genetics, Edinburgh*

Ichthyosis is an inherited condition in mice probably due to a single recessive mutant; its linkage relations are unknown. There is no evidence of incomplete penetrance.

A young ichthyotic mouse can be recognised when 4 days old by the reduced amount of pigment which should then be forming in the hairs under the skin. By 6 days it can be seen to be comparatively hairless; in this it differs from Naked, hairless, rhine and *hypotrichosis juvenilis* mice, which grow a normal first coat. At about 3 weeks the skin may become scaly and be sloughed off, especially from the neck, rump, tail and feet: this may interfere with the tail circulation and the distal part of the tail may become necrotic. Adult ichthyotics are always small, appear to suffer from respiratory disorders and may be mute: some grow a thin, downy coat. Males are cryptorchid and sterile, but one female has borne and suckled a small litter.

The condition was first found in the fourth generation of one of ten sublines of a stock maintained by sib-mating: it probably arose in that subline by spontaneous mutation.

## CYTOGENETICS OF MAIZE

D. G. CATCHESIDE, M. BLACKWOOD and J. N. HARTSHORNE
*Botany School, Cambridge*

## TWO NEW MUTANTS WITH NEUROLOGICAL ACTIONS IN THE HOUSE MOUSE

D. S. FALCONER
*Institute of Animal Genetics, Edinburgh*

"Trembler" is a dominant gene that arose by spontaneous mutation in 1946. It produces a spastic paralysis, particularly of the hind legs, and a generalised tremor in the adult, particularly of the head. The young are subject to convulsions upon stimulation. Females are fertile and rear their young well: males breed only irregularly. Homozygotes are indistinguishable from heterozygotes.

"Reeler" is a recessive gene that arose by spontaneous mutation in 1948. Homozygotes have a defect of balance that affects the hind quarters. When standing they sway from side to side, and when running they frequently fall right over on their side. They give a strong impression also of being mentally deficient. Males are sterile: one female has bred but failed to rear her young.

## LARGE AND SMALL MICE

D. S. FALCONER and J. W. B. KING
*Institute of Animal Genetics, Edinburgh*

CROSS-BREEDING EXPERIMENT.—Two strains of mice that had been independently selected for large size over many generations were obtained from H. D. Goodale of Mount Hope Farm, Massachusetts, and Prof. MacArthur of Toronto. These strains now give little response to continued selection for large size. The two strains were crossed and the $F_1$ gained about 8 per cent. from heterosis. Selection in both directions among the $F_1$ produced no change. It seems probable, therefore, that genetic variability was lacking in both the original strains and in the $F_1$. Selection in both directions is now being applied to the $F_2$, in which new genetic variability ought to be available.

SMALL MICE AND DWARFS.—A strain of mice selected for small size was also obtained from Prof. MacArthur. This strain has recently produced pathological dwarfs, a condition that appears to be determined by a single recessive gene. At the age of 6 weeks the weight of the dwarfs is about 21 per cent. of the weight of cross-bred large mice. The genetics and physiology of the dwarfs are being investigated.

## HETEROPLOID HYBRIDS IN NEWTS

M. FISCHBERG
*Animal Breeding and Genetics Research Organisation, Edinburgh*

Different nuclear and nucleo-cytoplasmic combinations can be effected in newt eggs by artificial fertilisation with sperm of other species. The maternal chromosomes are often either eliminated or doubled by hot (or cold) treatment of the egg immediately after fertilisation. By this means the balance between genomes of different species can be studied in combinations additional to the only two (homo- or heterozygote) possible in diploids.

The characters of *Triton alpestris* prevail in general over those of *T. palmatus*. If enucleated *palmatus* cytoplasm is combined with *alpestris* sperm a haploid embryo results which dies at a certain stage. A *palmatus* egg (cytoplasm+nucleus) combined with *alpestris* sperm gives a diploid hybrid which is mainly *alpestris* in character. If, however, a diploid *palmatus* egg (2 chromosome sets+cytoplasm) is combined with an *alpestris* sperm, a triploid hybrid arises with two "recessive" *palmatus* genomes+cytoplasm of *palmatus* and only one "dominant" genome of *alpestris*. Here therefore the two "recessive" genomes are stronger than the single "dominant" one, and the triploid hybrid resembles the "recessive" *palmatus*. By this means the effect of gene dosage on gene expression can be studied in vertebrates.

## DIFFERENTIAL SENSITIVITY OF CHROMOSOME REGIONS TO BREAKAGE BY NITROGEN MUSTARD

C. E. FORD
*M.R.C. Radiological Research Unit, Harwell, Berks.*

## THE GENETICS OF A COLONY OF THE MOTH *PANAXIA DOMINULA*

E. B. FORD, P. M. SHEPPARD and R. A. FISHER
*Department of Zoology, Oxford and Department of Genetics, Cambridge*

The spread of the *medionigra* gene in an isolated colony of this species has been studied for many years. Previous to 1928 it occupied 1·2 per cent. of available loci. By 1938 its frequency had risen to 9·2 and by 1940 to 11·1 per cent. Its frequency then suddenly dropped, and from 1941 to 1948 it has fluctuated between 6·8 and

4·0 per cent. of available loci. It is possible to make a detailed analysis of this situation since all three genotypes are recognisably distinct, while the gene-frequency and total numbers of the population in the locality can be calculated year by year. It appears that other genes responsible for easily recognised varieties are also being selected for in this population.

## GENETICAL RATIOS IN SAINFOIN

J. L. FYFE
*School of Agriculture, Cambridge*

Examples are shown of recessive types which segregate in first inbred progenies of common and giant sainfoin. The ratios obtained are tetrasomic, indicating that sainfoin is an autotetraploid.

## CYANOGENESIS IN WHITE CLOVER

J. L. FYFE and A. K. CHAKRAVARTY
*School of Agriculture, Cambridge*

Plants and picrate-paper tests are demonstrated to show the different types of cyanophoric and acyanophoric plants and the inheritance of the factors concerned.

## CYTOLOGY OF SPIROGYRA

M. B. E. GODWARD
*Queen Mary College, London*

(*a*) *Centromere organisation in Spirogyra.*
Photographs and preparations demonstrate that the different species may have :
(a) large chromosomes without a localised centromere,
(b) medium sized chromosomes with a localised centromere,
(c) minute chromosomes.

The existence of the type (c) having numerous minute chromosomes may mean that the large chromosomes of Type (a) are polycentric rather than possessed of "diffuse centromeres." Some evidence of only partially localised centromeres may be presented.

(*b*) *Variability in the nucleolar organiser in somatic cells of Spirogyra crassa* will be illustrated by photographs.

(*c*) *Variability in the activity of the nucleolar organisers, characteristic of different species of Spirogyra ;* with the resulting differences in the distribution of "nucleolar material" with regard to the chromosomes, in the different stages of mitosis. Photographs.

## MINOR SKELETAL VARIATIONS IN THE MOUSE

H. GRUNEBERG
*Department of Biometry, University College, London*

A survey of six pure lines and several less inbred stocks of mice has revealed many inherited skeletal variations, many of them of low penetrance. Some of these conditions have shown interaction with major skeletal genes for which they act as "modifiers." Four of these conditions are exhibited, namely :

(*a*) *Foramina transversaria imperfecta.*—Some foramina in the cervical vertebræ are transformed into ventrally open gutters. Almost completely penetrant in C57 Black ; also occurs in many wild populations.

(*b*) *Dyssymphysis atlantis et epistrophei.*—Abnormalities of fusion in the first two cervical vertebræ ; manifests in rather more than half the animals of the same stock.

(*c*) *Dystopia caudalis tuberculi anterioris.*—The tuberculum anterius of the sixth cervical vertebra is shifted on to the seventh cervical vertebra in about one-quarter of the animals of the X stock.

(*d*) *Variations of the vertebra prominens.*—In all mouse stocks examined, the processus spinosus of the second thoracic vertebra may vary from a large spatula-shaped affair down to complete absence. The distribution is very different from stock to stock, with peaks either near one end of the distribution, or near the other, or near the middle, or near both ends.

## REVIEW OF THE WORK DONE IN THE GENETICS OF *DROSOPHILA SUBOBSCURA* DURING THE LAST TEN YEARS

J. B. S. HALDANE *et al.*
*Department of Biometry, University College, London*

Demonstration of photographic, diagrammatic and live material.

## RESULTS OBTAINED BY A NEW METHOD OF DIAGNOSING TASTE THRESHOLDS

H. HARRIS and H. KALMUS
*Galton Laboratory, University College, London*

## CHANGES ACCOMPANYING SELECTION FOR PLEIOCOTYLY

G. HASKELL
*John Innes Horticultural Institution*

The demonstration shows that polygenic selection, using seedling cotyledon number as selection criterion, produces correlated disturbances in the normal, balanced condition of adult Dicotyledonous plants. These are comparable with the changes following chaetæ selection in *Drosophila melanogaster* observed by Mather, Wigan and Harrison.

The gamut of pleicotyly from monocotyly to tetracotyly is illustrated and pedigrees are given showing the results of pleicotyly selection in outbreeding species (Cruciferæ), partly outbreeding species (Umbelliferæ) and inbreeding species (Solanaceæ). Selection has increased the frequency and range of pleicotyly in the first two groups but so far has been ineffectual with inbreeding species. Environmental influences on cotyledon manifestations are also shown.

Tables of various economic forms of *Brassica oleracea* give the comparative morphology between dicot controls and plants from selected pleiocotylous seedlings grown to seed in isolation groups. The controls were normal with few exceptions, while some selections gave pleiocot seedlings which grew either into normal or abnormal adults. Photographs show the types of abnormalities which accompanied pleiocotyly selection in brassicas and other species. Although the disturbed growth may be due to pleiotropic effects of genes controlling pleiocotyly, lowered fertility and reduced vigour indicate other genes are also involved in producing these correlated changes.

## DERMAL RIDGE COUNTING AS A PRELIMINARY TO GENETICAL STUDIES

S. B. HOLT
*Galton Laboratory, University College, London*

## $P^{32}$ AUTORADIOGRAPHS OF MOUSE TESTIS

A. HOWARD and S. R. PELC
*Medical Research Council, Radiotherapeutic Research Unit, London*

Young male mice were injected with 200*u*C of radioactive phosphorus ($P^{32}$). After 2, 5, 9, 12 and 18 days testes were removed and preparations made as follows: (1) 5*u* sections fixed in acetic acid-alcohol; (2) smears fixed likewise; (3) squashes fixed in methyl alcohol and spread and washed in 45 per cent. acetic acid; (4) whole tubule mounts fixed in methyl alcohol. Autoradiographs were prepared by the stripping film technique: exposure was for 7-28 days. In smears and sections autoradiographs appeared at 9 days and more intensely at 2 days above interstitial tissue, associated with Leydig cells. Whole tubule mounts at 2 days show patches along their length at approximately 3 mm. intervals. At 9 days and later autoradiographs were seen above Sertoli cells and above spermatocytes at late diplotene and thereafter. In squashes autoradiographs of chromosomes were found at late diplotene and diakinesis; none were found at earlier meiotic stages. Mature sperm in squashes show no autoradiograph, which is, however, present (in 5 to 18 day mice) above immature sperm in smears and sections, presumably due to Sertoli cell cytoplasm.

Points arising from the above are (1) Leydig cells apparently accumulate acid-insoluble phosphorus to a large extent. This may be due to their supposed hormonal function. Patches of autoradiograph observed along the tubules may be of similar origin and/or may be associated with the spermatogenic wave. (2) A schedule of the time taken by different stages of spermatogenesis can be attempted from results on a series of mice.

H. W. HOWARD
*School of Agriculture, Cambridge*

"Genetics of *Armadillidium vulgare*."—Dominant and recessive red animals occur in natural populations. The two genes may be at the same locus.

"Cytology of *Avena sativa*."—$F_1$ hybrids between spring varieties and winter varieties of *Avena sativa* have a high frequency of univalent formation which is only slightly less than the frequency for the cross winter variety of *Avena sativa* × *A. fatua*.

"Grafting experiments with potatoes."—The short-day species *S. demissum* grafted with scions of the long-day adapted variety Epicure sets a larger yield of tubers than when not grafted. There is some effect of the grafting in the following year on plants grown from tubers of the grafted stock.

"Chromosome numbers and systematic in the cruciferous genera *Nasturtium*, *Rorippa* and *Cardamine*."—Diploid and tetraploid forms occur in several species of these three genera.

## LETTERS OF THE EARLY MENDELIANS

R. HURST
*Horsham, Sussex*

Mrs Hurst will give a short explanatory talk, the place and time of which will be announced at the meeting.

## SEROLOGICAL TYPING OF PEDIGREE MATERIAL FOR LINKAGE STUDIES

S. LAWLER and L. S. PENROSE
*Galton Laboratory, University College, London*

## INCREASED VARIABILITY AFTER X-IRRADIATION IN INBRED LINES

D. LEWIS and B. J. HARRISON
*John Innes Horticultural Institution*

## SPECIES CROSSES IN ANTIRRHINUM

K. MATHER and A. VINES
*Department of Genetics, University of Birmingham*

(*a*) *Cleistogamy.*—In an $F_2$ from *Antirrhinum majus* × *A. glutinosum* two plants were found to have flowers which failed to open. Their foliage was also of an unusual type.

This cleistogamy is heritable, though not simply so. Intermediate forms appear. Fully cleistogamous flowers set seed naturally only by self-pollination—bees cannot penetrate into the flower. Thus two species, one of them an obligatory crossbreeder and the other regularly showing some crossbreeding, have between them all the genetical material for producing an obligatorily inbreeding form.

(*b*) *Flower Colour.*—*A. glutinosum* has a little anthocyanin colour but its flowers appear acyanic (ivory white) to casual inspection. This whiteness is not due to any of the available major gene mutations which are known to reduce or remove anthocyanin pigments in *A. majus*. At least two genes of more major action are involved in the species difference and there is also evidence of a polygenic system affecting flower colour.

In the $F_2$, plants with the flower colour of *glutinosum* have not been observed so far, although plants with acyanic flowers do occur in the expected proportions when the *majus* parent introduces one of the mutant genes having this effect.

## GENETICS OF *BOMBARDIA LUNATA*

M. J. MATHIESON
*Botany School, Cambridge*

## SOME X-RAY-INDUCED MUTATIONS IN *COPRINUS LAGOPUS*

U. MITTWOCH
*Galton Laboratory, University College, London*

## A NEW INHERITED POLYDACTYLY IN THE HOUSE MOUSE

R. S. PHILLIPS
*Institute of Animal Genetics, Edinburgh*

Preaxial (1st toe) polydactyly of the hind feet in mice has been reported by numerous workers. Postaxial (Vth toe) polydactyly of the fore feet has been described by Strong (1934). Holt (1945) found two animals with duplication of the Vth toe of the hind feet, but was unable to report on the inheritance.

Animals with a complete or partial duplication of the fifth toe of the hind foot, have recently been found on inbreeding mice obtained from a fancier. This condition is associated with a postnumional protuberance on the fore foot, resembling that described by Strong (1934). The abnormalities were found to be heritable, but the inheritance does not follow a simple Mendelian pattern. On outcrossing to various inbred lines (CBA, C.57 black, A strain, etc.) the fore foot condition was found in about one-fifth of the $F_1$ mice; but the hind foot condition only reappeared after backcrossing to the original stock. When the fore foot abnormality is unilateral, the right side is more often affected than the left.

## "BALANCED" HETEROKARYONS IN *ASPERGILLUS NIDULANS*

G. PONTECORVO
*Department of Genetics, University, Glasgow*

Demonstration of heterokaryons between mutant strains differing in the colour of the conidia as well as in growth-factor requirements. Grown on agar medium not supplying the growth factors, these heterokaryons are "balanced" (permanent). Both colours of the parental strains can be seen in different chains of conidia arising from individual fruiting heads. Heterokaryon (intracellular syntrophism) and component strains (intercellular syntrophism) are in equilibrium. The equilibrium varies with the growth factor requirements of the component strains and changes as the growth factors accumulate in the medium, presumably from autolysis of older cells. In the case of vitamin requirements the equilibrium involves a small proportion of heterokaryotic mycelium and a high proportion of component strains; with less diffusible factors, and/or growth factors required in large amounts, the heterokaryon is favoured. During growth of a heterokaryotic colony addition to the solid medium of the required growth factors shifts the equilibrium in favour of either or both constituent strains. The shift is clearly seen by the change in colour in the part of the colony grown after such addition.

## GENETICS OF THE HOMOTHALLIC ASCOMYCETE *ASPERGILLUS NIDULANS*

G. PONTECORVO, E. FORBES and O. B. ADAM
*Department of Genetics, University, Glasgow*

Heterokaryons between strains differing in three or more pairs of characters are established.

The ascospores from these heterokaryons are of parental type, mainly from self karyogamy, and biochemical or morphological recombinant, all from cross karyogamy. In any one cross certain classes of recombinants (morphological or biochemical) are taken as markers to study segregation and recombinations of other genes in these classes. The "morphological" recombinants (colours of the conidia) are identifiable by inspection; the biochemical ones by using selective media on which only certain types of recombinants can grow (*cf.* Lederberg).

Ten loci have been identified, two affecting the colour of the conidia and eight affecting growth factor requirements; four loci belong to one linkage group. An insertional translocation is present in one strain.

Preliminary work on the cytology of the species suggests the haploid number of chromosomes to be four.

## PRODUCTION OF MUTANTS IN *ASPERGILLUS NIDULANS*

G. PONTECORVO and J. A. ROPER
*Department of Genetics, University, Glasgow*

(*a*) Production of the mutants following mutagenic treatment.
(*b*) Identification of the growth factor requirements of "biochemical" mutants; details of "auxanographic" techniques.
(*c*) Detailed biochemical characterisation of a number of arginine-requiring, lysine-requiring and nicotinic acid-requiring mutants.

## PEDIGREES ILLUSTRATING THE INHERITANCE OF HUMAN BLOOD GROUPS

R. R. RACE, S. D. LAWLER, D. BERTINSHAW and H. HOLT
*Medical Research Council, Blood Group Research Unit, Lister Institute, London*

With the exception of the Lewis gene all known blood group genes express themselves in the single dose of the heterozygote; expression in the homozygote is usually recognisably greater.

Pedigrees are shown illustrating the inheritance of the $A_1$ $A_2$ B O, the MNS, the Rh, the Lutheran, the Kell and the rare Levay blood groups.

The Lewis gene, in the adult, has to be present in the homozygous state if the Lewis antigen is to be found on the red cells. All persons so far tested whose red cells carry the Lewis antigen are salivary non-secretors of the ABO blood group substances. The inability to secrete these substances in the saliva has long been recognised as a recessive character. Pedigrees are shown of families tested for these two characters.

## THE INHERITANCE OF BODY SIZE IN *DROSOPHILA MELANOGASTER*

E. C. R. REEVE and F. W. ROBERTSON
*Institute of Animal Genetics, Edinburgh*

A study is being made of the inheritance of body size in *Drosophila*. Extensive selection experiments have been carried out for wing and thorax length, and strains have been produced which differ greatly in size. These differences are due primarily to changes in cell size rather than cell number. Other morphological and physiological changes are being examined in detail. Selection for each character has altered both the ratio of the two dimensions and the relative sizes of the sexes.

Using progeny tests of factorial design and crosses to standard strains, the genetic situation in the various lines is being analysed, to assess the extent and nature of the free variation remaining at different stages in selection ; to examine the genetic differences between lines of different size and to study the basis for the heterosis which has appeared in certain crosses.

## CYTOLOGICAL MAPPING OF MAMMALIAN CHROMOSOMES

B. M. SLIZYNSKI
*Institute of Animal Genetics, Edinburgh*

Mammalian testes offer an opportunity for detailed morphological studies of meiotic prophase chromosomes. A method of aceto-carmine rapid or delayed fixation followed by squashing and/or smearing of small bits of tubules, followed by treatment with Feulgen reagent or by staining with basic Fuchsin after hydrolysis, is used for obtaining slides. On such slides the spread prophase chromosomes can be studied in detail and cytological maps can be drawn. Structural patterns which are typical for each chromosome make the exact identification of any chromosome possible, though difficult. A preliminary map of the complete set of mouse chromosomes has been made and a similar map of bull chromosomes is well advanced. In full pachytene the over-all length of mouse chromosomes is about 170 microns. Slides and photographs show typical preparations from mouse testis and from bull testis.

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